UNIVERSIDADE TÉCNICA DE LISBOA

Faculdade de Medicina Veterinária

"SKIN PRICK TESTS – PRELIMINARY EVALUATION OF THIS TECHNIQUE FOR THE DIAGNOSIS OF CANINE ATOPIC DERMATITIS SENSITIZATION"

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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Dedicado à minha família e ao meu namorado

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I want to thank Prof. Ana Mafalda Lourenço Martins and Dr. Joana Vidal Pontes, for all the knowledge you have given me, for the enormous patience and for giving me a second chance.

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Last but not least, to my boyfriend thank you so much for all the love and support through all these years.

To all of you… Thank you very much!

Abstract - “Skin Prick Tests – Preliminary evaluation of this technique for the diagnosis of canine atopic dermatitis sensitization”

Canine atopic dermatitis (CAD) is a multifactorial disease involving a type I hypersensitivity to allergens, cutaneous barrier defects, microbial infections and other flare factors. It is “an inflammatory and pruritic skin disease, with genetic predisposition and characteristic clinical features associated with IgE antibodies most commonly directed against environmental allergens”.

The clinical signs of CAD frequently manifest from 6 months to 3 years of age. Some breeds, such as Bull Terrier, Cocker Spaniel, English Bulldog, German Shepherd Dog, Golden Retriever, Labrador Retriever, Pug, Shar Pei, West Highland White Terrier, Yorkshire Terrier, among others, seem to be at a higher risk of developing the disease.

The lesions are typically distributed in the periocular skin, muzzle, ears, interdigital area, flexural joints of the extremities, axillae, abdomen, groin and perineum. The first clinical manifestation is usually pruritus with erythema, followed by secondary lesions, such as excoriations, self-induced alopecia, dry hair, hyperpigmentation, scaling and lichenification, which reflect chronic pruritus and inflammation, concurrent secondary infections and bacterial overgrowth.

The diagnosis of the disease is based on the anamnesis, clinical signs, dermatological examination, exclusion of other pruritic skin diseases and allergy testing, with *in vivo* techniques, such as Intradermal Tests (IDT) or Skin Prick Tests (SPT), and *in vitro* methods, such as, allergen-specific IgE measurements.

CAD has no cure, however it is possible to be controlled with multifactorial treatment, which may include allergen avoidance measures, allergen-specific immunotherapy (ASIT), improvement of the skin barrier function and anti-inflammatory therapy.

The objective of this study was to determine if SPT technique was doable in dogs. Also, it was important to determine whether or not the standardized concentrations of the allergens used for human patients would cause irritant false positive skin reactions in healthy non-atopic dogs. Therefore, 22 healthy non-atopic dogs were tested with 15 aqueous allergens commercially available for use in human medicine.

The results showed that all dogs had negative skin reactions to the concentrations available for each allergen tested.

It was possible to conclude that the SPT were not only doable but they showed many advantages versus IDT, such as simplicity, rapidity, less discomfort, less irritation and safety, and also, that the concentrations used did not cause irritant false positive skin reactions in healthy dogs.

Keywords: Canine atopic dermatitis, skin prick tests, allergens

Resumo – "Testes Cutâneos *Prick* – Avaliação preliminar desta técnica no diagnóstico de sensibilização na dermatite atópica canina."

A dermatite atópica canina (DAC) é uma doença multifactorial que envolve uma hipersensibilidade de tipo I a alergénios, alterações da barreira cutânea, infecções microbianas entre outros factores ambientais. Constitui uma doença cutânea inflamatória e prurítica, com predisposição genética e sinais clínicos característicos associados a anticorpos IgE mais frequentemente direccionados contra alergénios ambientais.

Os sinais clínicos da DAC manifestam-se frequentemente entre os 6 meses e os 3 anos de idade. Algumas raças, como Bull Terrier, Cocker Spaniel, Bulldog Inglês, Pastor Alemão, Golden Retriever, Labrador Retriever, Pug, Shar Pei, West Highland White Terrier, Yorkshire Terrier, entre outros, parecem estar em maior risco de desenvolver a doença.

Existe uma distribuição lesional típica em certas regiões do corpo, como região periocular, focinho, pavilhões auriculares, zona interdigital, articulações das extremidades, axilas, abdómen, virilhas e períneo. O primeiro sinal clínico é o prurido com eritema, seguido de lesões secundárias, como escoriações, alopécia auto-induzida, pêlo de má qualidade, hiperpigmentação, seborreia seca e liquenificação, que reflectem prurido e inflamação crónicas, infecções secundárias concomitantes e sobrecrescimento bacteriano.

O diagnóstico da doença é baseado na anamnese, sinais clínicos, exame dermatológico, exclusão de outras doenças de pele pruríticas e testes alérgicos, por técnicas *in vivo*, testes Intradérmicos ou testes *Prick* (também denominados "por picada"), ou por métodos *in vitro*, medição de IgE específica.

A DAC não tem cura, no entanto, pode ser controlada através de um tratamento multifactorial, que pode incluir medidas de evicção alergénica, imunoterapia alergénio-específica, melhoramento da função da barreira cutânea e terapêutica anti-inflamatória.

Este estudo teve como objectivo determinar se a técnica do teste cutâneo *prick* era exequível em cães. Foi, também, importante determinar se as concentrações padronizadas dos alergénios para os doentes humanos, provocavam reacções cutâneas falso positivas irritantes em cães saudáveis não-atópicos. Para isso, 22 cães saudáveis não-atópicos foram testados para 15 alergénios aquosos disponíveis comercialmente para utilização em medicina humana.

Os resultados mostraram que todos os cães tiveram reacções cutâneas negativas para as concentrações disponíveis para cada alergénio testado.

Foi possível concluir que os testes *Prick* não só eram exequíveis em cães, como mostraram muitas vantagens em comparação com os testes Intradérmicos, como simplicidade, rapidez, menor desconforto, menor irritação e maior segurança, e também, que as concentrações utilizadas não causaram reacções cutâneas falso positivas irritantes em cães saudáveis.

Palavras-chave: Dermatite atópica canina, testes cutâneos *Prick*, alergénios

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List of abbreviations

AD – Atopic Dermatitis
ALD – “Atopic-Like Dermatitis”
Alt a – *Alternaria alternata*
AMPs – Antimicrobial Peptides
ASIT – Allergen-specific Immunotherapy
CAD – Canine Atopic Dermatitis
CADESI – Canine Atopic Dermatitis Extent and Severity Index
CsA – Cyclosporine A
DAC – Dermatite Atópica Canina
Dac g – *Dactylis glomerata*
Der f – *Dermatophagoides farinae*
Der p – *Dermatophagoides pteronyssinus*
ELISA – Enzyme-Linked Immunosorbent Assay
EFA – Essential Fatty Acids
EPA – Eicosapentaenoic Acid
FLG – Filaggrin Gene
GLA – Gamma-Linolenic Acid
HDM – House Dust Mite
HEP – Histamine Equivalent Prick
HPA – Hypothalamic-Pituitary-Adrenal
ICU – Intensive Care Unit
IDT – Intradermal Tests
IgA – Immunoglobulin A
IgE – Immunoglobulin E
IgG – Immunoglobulin G
IL – Interleukin
ITC – Irritant Threshold Concentration
ITFCAD – International Task Force on Canine Atopic Dermatitis
Lep d – *Lepidoglyphus destructor*
Lol p – *Lolium perenne*
MHC – Major Histocompatibility Complex
Ole e – *Olea europaea*
Phl p – *Phleum pratense*
PMN – Polymorphonuclear
PNU/ml – Protein Nitrogen Units per milliliter
PRR – Pattern-recognition Receptors
RAST – Radioallergosorbent Test

RNA – Ribonucleic Acid

SC – Stratum Corneum

Sec c – *Secale cereale*

sIgE – Specific Immunoglobulin E

SPT – Skin Prick Tests

TEWL – Transepidermal Water Loss

TGF-β – Transformation Growth Factor-β

Th – T-helper

TLR- Toll-like Receptor

TNF-α – Tumor Necrosis Factor-α

Treg – T-regulatory

UK – United Kingdom

WHO – World Health Organization

w/v – weight/volume

γ-IFN – γ-Interferon

mm – millimeters

mg/kg – milligrams per kilogram of body weight

µg/kg – micrograms per kilogram of body weight

mg/ml – milligrams per milliliter

µg/ml – micrograms per milliliter

I. Introduction and Description of activities

1. Introduction

The internship was conducted at the Teaching Hospital of the Faculty of Veterinary Medicine, Technical University of Lisbon, from February 1 and July 31, 2011, in a total of 1070 hours of work, under the scientific supervision of Professor Ana Mafalda Lourenço Martins and Dr. Joana Vidal Pontes.

During the internship, several activities have been developed with the purpose of applying and integrating concepts and procedures already acquired giving the opportunity to acquire and learn new ones in the clinical practice.

My interest in Dermatology determined the choice of Professor Ana Mafalda Lourenço Martins, that suggested my participation in a project related to CAD, which would result in this Master's degree dissertation.

Also, under the supervision of Dr. Joana Pontes, I had the opportunity to learn many techniques and procedures in the areas of Internal Medicine and Ultrasonography.

I chose to write my master's degree dissertation in English, in spite of the fact that it was not mandatory, to allow wide access of this study, including at an international level, which would be difficult if written in Portuguese.

1.1. Description of activities

The activities at the Teaching Hospital of the Faculty of Veterinary Medicine, Technical University of Lisbon, are divided into Internal Medicine, Surgery, Intensive Care Unit (ICU) and Imaging. There are also specialty consultations in the areas of Dermatology, Neurology, Orthopedics, Ophthalmology, Endocrinology, Cardiology, Animal Behavior and New Companion Animals.

The services of Internal Medicine, Surgery and ICU are available 24 hours a day, whereas the specialty consultations and Imaging, except for radiography, work under previous appointments.

The intern in this Hospital had a schedule to follow, in which he must rotate through the various areas mentioned above, and in each had different activities.

Internal Medicine

In this area, the intern is supposed to receive the patient, collect the clinical history and anamnesis, perform the clinical examination, and discuss with the attending veterinarian the differential diagnosis to the problems presented by the patient, the adequate diagnostic

complementary exams, define a definitive diagnosis, if possible, and adequate therapy for each particular case.

In this area, the intern also performs or helps in the procedures involving several diagnostic exams, such as collecting blood samples, cytology, collecting urine samples, radiography, ultrasonography, echocardiograms, computerized tomography (CT) and electrocardiograms. Other activities include venous catheterization, cleaning and observation of the ear canal, cleaning of wounds, drug administration, vaccination, amongst others.

Imaging

In this area, the intern performs and interprets radiographic exams, ultrasonographic exams, computerized tomography and endoscopy scans of first opinion and referred clinical cases. In this service, the student also participates in the contention and positioning of the animals, and also in the anesthesia, if necessary.

In the area of imaging, the student spent most of the time in ultrasonography and had the opportunity to perform several ultrasonographic examinations, in which it was possible to diagnose several clinical conditions, such as foreign bodies, localized tumors, amongst others, and also help in ultrasound guided fine needle aspiration punctures.

Surgery

Here, the intern performs the pre-surgery preparation of the animal that includes sedation, venous catheterization, intubation, trichotomy and disinfection of the surgical area, and monitoring the animal vital signs.

In the actual surgery, the intern can perform several activities, as the surgeon assistant or anesthesiologist, which provides the opportunity to follow or actively assist in the surgery.

After the surgery, the intern must monitor the patient until its condition is stable, and post-surgery consultations to change the surgical dressings, remove stitches, and observe the surgical wound.

ICU

In the ICU, the intern has rounds of 12 or 24 hours, in which he was responsible for monitoring of vital signs of the animals (heart rate, respiratory frequency, mucosa, capillary repletion time, pulse, temperature, cardiac and pulmonary auscultation), administration of medication, feeding and hygiene care.

In this service, the intern also has to collect blood and/or urine samples, perform venous catheterization, vesicular washings, enema, simple dressings, and monitor and care the animals in the oxygen chamber.

Dermatology

Of the specialty consultations, the student had the closest contact with the service of Dermatology, due to the particular interest in this area. In this service, under the supervision of Prof. Ana Mafalda Lourenço Martins, he has been given the opportunity to observe and collaborate in the Dermatology consultations. The intern collected the clinical history and anamnesis of the patient, and performed the physical and dermatological examinations, which included performing and interpreting cutaneous and auricular cytology, trichogram, administration of allergen-specific immunotherapy (ASIT) in patients and collecting blood samples for serology. He was also provided with the opportunity to follow and perform the techniques of video otoscopy and cutaneous biopsy.

The clinical cases observed in these consultations consisted mostly of Canine Atopic Dermatitis, with or without *Malassezia* and/or *Staphylococcus* infections, otitis externa associated with CAD and demodectic mange.

II. Atopic Dermatitis

1. Definition

"Atopic dermatitis (AD) is a multifactorial disease involving allergies, cutaneous barrier defects, microbial infections and other flare factors" (Nuttall, 2008). It is a common dermatosis of dogs defined as "a genetically-predisposed inflammatory and pruritic skin disease with characteristic clinical features associated with IgE antibodies most commonly directed against environmental allergens" (Halliwell, 2006).
It is now recognized a parallel condition termed "atopic-like dermatitis" (ALD) that must be differentiated from AD. Canine atopic-like dermatitis is defined as "an inflammatory and pruritic skin disease with clinical features identical to those seen in canine atopic dermatitis in which an IgE response to environmental or other allergens cannot be documented" (Halliwell, 2006).

2. Pathogenesis

2.1. Skin barrier

The protective role for an intact skin barrier is performed by the most superficial epidermal layer, the stratum corneum (SC), which is composed by cornified keratinocytes, known as corneocytes, surrounded by complex lipid lamellae, enriched in ceramides, cholesterol and free fatty acids. The SC protects from transepidermal water loss (TEWL) and penetration of exogenous molecules, like allergens, irritants and toxic agents (Olivry & Hill, 2001; Elias, 2008; Olivry, 2011).
There is increasing evidence that a skin barrier defect is present in dogs with atopic dermatitis, like has been shown in human atopic dermatitis (Olivry, 2011).
In humans, this barrier dysfunction is thought to be due to loss-of-function mutations in the filaggrin (an intracellular protein) gene *FLG*, which would predispose for the development of AD, by altering corneocyte shape and disrupting the organization of the extracellular lamellar bilayer. One of the effects of FLG deficiency is the decreasing of the SC hydration, which leads to increased TEWL. Deficiency in FLG products could increase pH of the SC in AD, inducing multiple serine proteases that could precipitate downstream structural and functional alterations. Currently, the "outside-inside-outside" theory for the pathogenesis of human AD defends that a genetic skin barrier defect predisposes to atopic cutaneous inflammation, by inducing primary cytokines, which are the primary contributors to inflammation, and antigen ingress through a defective barrier, that would be the second

cause of inflammation in AD (Olivry & Hill, 2001; Leung & Bieber, 2003; Elias, 2008; Elias & Schmuth, 2009; Olivry, 2011).

The measurement of TEWL is not the optimal method to evaluate skin barrier function in dogs, because it varies widely from day-to-day, site-to-site and dog-to-dog. However, in dogs with AD, this method showed that TEWL seems higher in lesional AD skin than non-lesional AD skin or normal skin, and allergen challenge increases these values (Olivry & Hill, 2001; Leung & Bieber, 2003; Elias, 2008; Elias & Schmuth, 2009; Olivry, 2011).

The antimicrobial barrier also seems to be compromised because of *Staphylococcus aureus* and/or *Malassezia* yeast colonization, which favors secondary infections. This colonization can further aggravate the barrier abnormality (Barata, 1999; Olivry & Hill, 2001; Leung & Bieber, 2003; Elias, 2008; Elias, Hatano & Williams, 2008; Cork et al., 2009; Elias & Schmuth, 2009; Addor & Aoki, 2010; Olivry, 2011).

Other changes found in the skin of dogs with AD include:

- Disorganized corneocytes with intercellular spaces; thinner, less common and disorganized lipid lamellae in non-lesional AD skin;
- Higher disorganization of corneocytes and lipid lamellae with lamellar bodies within corneocytes after allergen challenge;
- Lower quantity and proportion of free and protein-bound SC ceramides as well as several ceramide subclasses;
- Higher quantity of free and protein-bound glucosylceramides, which suggest an abnormal ceramide metabolic pathway;
- Lower quantity, in the skin and plasma, and higher degradation of sphingosine-1-phosphate (natural anti-inflammatory ceramide-degradation product);
- Absent or abhorrent expression of C-terminal filaggrin in some dogs with AD, and allergen challenge reduce filaggrin transcription, which can be suggestive of FLG mutations.

It is not yet known if only some or all dogs with AD have similar skin barrier defects and if these are primary (of genetic origin) and/or secondary to atopic skin inflammation (Taïeb, 1999, Olivry & Hill, 2001; Novak, Bieber & Leung, 2003; Bouwstra & Ponec, 2006; Bieber, 2008; Elias, 2008; Yosipovitch & Papoiu, 2008; Elias & Schmuth, 2009; Olivry, 2011). It is also unknown if the correction of this barrier defect, with nutritional lipid supplementation and/or topical lipid application as any therapeutic benefit. However, one study shows that nutritional supplementation for 9 weeks with pantothenic acid, nicotinamide and pyridoxine (water-soluble vitamins); histidine and proline (amino acids); inositol and choline (vitamin B complex) can have a stimulatory effect on lipid synthesis and significantly decrease TEWL (Watson et al., 2006).

2.2. Immunopathogenesis

The pathogenesis of AD is extremely complex, and can be categorized by three major components:

1) Defects in barrier function (has stated above);
2) Defects in innate immunity;
3) Defects in acquired immunity.

The immune system protects from pathogens, as well as initiates the repair process after injury or trauma. This is due to the close interaction between the innate and adaptive immune pathways (Novak et al., 2003; De Benedetto, Agnihothri, McGirt, Bankova & Beck, 2009; Halliwell, 2009).

The innate immune system is the first line of defense against environmental insults. These insults are sensed by a group of receptors, known as pattern-recognition receptors (PRRs), that include transmembrane and intracellular receptors, such as the Toll-like receptors (TLRs). PRRs recognize molecular patterns of pathogens, known as “pathogen-associated molecular patterns”, like bacterial cell-wall components, fungal cell wall, viral double-stranded RNA molecules. The activation of PRR results in the production of cytokines, chemokines, and antimicrobial peptides (AMPs), and in the activation of immune cells (immature dendritic cells, natural killer cells and neutrophils). Defects in the Toll-like receptors, especially TLR2, has been shown in patients with AD, which can be an explanation for higher susceptibility to skin infections (Novak et al, 2003; De Benedetto et al., 2009; Halliwell, 2009) .

Histopathology of human and canine patients with AD, have shown an absence or low quantity of polymorphonuclear (PMN) cells, even in presence of intense scratching and colonization/infection with *S. aureus.* This can be due to a chemotactic defect in PMN cells in AD. PMN cell activities seem to be particularly impaired in AD patients with concomitant bacterial infections. Some studies showed that the upregulation of the leukocyte adhesion molecule CD11b is decreased in humans with AD, which can also explain the defect in the quantity of neutrophils. PMNs are critical cells in the primary response against all pathogens, so it is expected that a defect in the recruitment of these cells renders the skin of patients with AD more susceptible to secondary infections (Novak et al, 2003; De Benedetto et al., 2009; Halliwell, 2009).

AMPs are also thought to be defective in AD. AMPs directly kill a large spectrum of microorganisms including Gram-positive and Gram-negative bacteria, fungi and some viruses. The antimicrobial properties derive from their ability to integrate and disrupt the cellular membrane of the organism. These peptides can modulate host immune responses, like leukocyte chemotaxis and activation of PRRs. In normal conditions, AMPs are present at low or undetectable levels, but are induced after injury or inflammation. In human patients the number of AMPs after injury has been shown to be defective, maybe in part because some

organisms, like *S. aureus,* produce proteases or toxins, that interfere with host AMPs. The AMPs also act as a link between innate and adaptive immune responses. However, in canine patients, studies on AMPs have not been conclusive (De Benedetto et al., 2009; Halliwell, 2009).

Skin biopsies of dogs with AD show epidermal Langerhans cell hyperplasia with IgE antibodies, that are responsible for allergen capture and processing. Increased numbers of dermal dendritic cells, with similar functions, are also found. Mast cell hyperplasia has also been shown, however, there are not significant differences in the mast cell density. Lymphocytes are frequent, especially T cells (Damsgaard, Olesen, Sørensen, Thestrup-Pedersen & Schiøtz, 1997; Novak et al., 2003; De Benedetto et al., 2009; Mrabet-Dahbi & Maurer, 2010).

T cell responses can be of two types:

- Th1 response, associated with IL-2, IL-12, IL-18 and γ-IFN, expressed as cell-mediated immunity;
- Th2 response, associated with IL-4, IL-5, IL-6 and IL-13, which facilitates antibody production, including IgE.

Several studies suggest that a Th2 response is associated with the acute phase of AD, while a Th1 response occurs in the chronic phase, where secondary infection is superimposed (Novak et al., 2003; De Benedetto et al., 2009; Halliwell, 2009; Mrabet-Dahbi & Maurer, 2010).

Mast cell derived mediators, such as, histamine, proteases and serotonin, clearly have an important role in canine AD. Nevertheless, the poor effect of antihistamines as a sole treatment suggests that other mediators must have more pronounced pruritogenic and inflammatory effects, like membrane-derived mediators, especially the leukotrienes (Damsgaard et al., 1997; Marsella & Olivry, 2001; De Benedetto et al., 2009; Mrabet-Dahbi & Maurer, 2010).

Bacterial overgrowth and pyoderma is very common in canine AD. One reason for this is the enhanced ability of canine *Staphylococcus* species to adhere to corneocytes of atopic dogs. *Malassezia* overgrowth is also a common feature in AD. An IgE response can be an important factor in the disease process (Novak et al., 2003; De Benedetto et al., 2009; Halliwell, 2009; Lloyd, 2009).

The current theory on the pathogenesis of canine AD states that in the acute phase of the disease, epidermal barrier defects are thought to facilitate contact of environmental (and possibly microbial) allergens with epidermal immune cells. Epidermal antigen-presenting cells (Langerhans cells) capture allergens with allergen-specific IgE, and then migrate to the dermis and regional lymph nodes, where a immune Th2 response to the allergen is developed. Immunoglobulin E-coated dermal mast cells release histamine, proteases, chemokines and cytokines, following contact with allergens. There is an early influx of

granulocytes (neutrophils and eosinophils), allergen-specific T-lymphocytes and dermal dendritic cells. There is a continuous cycle of chemokine release that leads to the influx and activation of leucocytes and to the release of additional pro-inflammatory mediators. In the chronic phase, a concomitant Th1 response occurs with γ-IFN prominent. This is compound by secondary infections, which stimulate further Th1 responses. The failure to downregulate these immune mechanisms is followed by a continuation of the immune responses and resultant inflammation (Barata, 1999; Leung & Bieber, 2003; Novak et al., 2003; Bieber, 2008; De Benedetto et al., 2009; Halliwell, 2009; Lloyd, 2009; Mrabet-Dahbi & Maurer, 2010; Okada, Kuhn, Feillet & Bach, 2010).

In human medicine there is the concept of a T-regulatory (Treg) cell-mediated immune suppression. There are two known main groups of Treg cells: the natural Treg cells, with a $CD4^{+}CD25^{+}$ phenotype, and the adaptive Treg or T-regulatory type 1 (Tr1), responsible for the secretion of IL-10 with or without TGF-β. These cells are thought to have an ability to suppress proliferation of effector T cells. It has been speculated that migration of increased numbers of Treg cells or induction of their local proliferation to the inflamed area, could have a beneficial effect in the treatment of several inflammatory diseases, like allergy (Verhagen et al., 2006).

However, according to a study performed by Verhagen et al. (2006), that evaluated the presence of Treg cells, IL-10 and TGF-β cytokines, and also their capability to suppress T-cell effector functions in AD skin, it was shown that IL-10-secreting Tr1 cells, but not CD4+CD25+ T cells, infiltrate lesional AD skin. These results demonstrate an imbalance in T-cell regulation, which can be an explanation for the continuing inflammation in AD skin. More studies are needed to evaluate if this is also true for canine AD.

3. Incidence and Prevalence

There is an increasing incidence of atopic dermatitis in humans, particularly in developed countries. Even though there is a genetic predisposition to the development of this disease, the rapid rise in incidence is suspected to be caused by environmental rather than genetic factors (Hillier & Griffin, 2001).

According to the “hygiene hypothesis”, for human patients, changes in lifestyle in industrialized countries led to the decrease of infections and are associated with the rise of allergic diseases. This is sustained by the idea that the interaction with some infectious agents are able to protect against a large spectrum of immune-related disorders. The measures in public health after the industrial revolution, as decontamination of the water supply, pasteurization and sterilization of milk and other food products, vaccination and the wide use of antibiotics, can explain the emergence of allergic and autoimmune diseases in developed countries. To explain the protective influence of infectious organisms from

immunological disorders, a Th1-Th2 deviation mechanism has been used. It was suggested that, in industrialized countries, the lack of microbial burden, that favors a Th1-biased immunity, redirects towards a Th2 immune response, and predisposes allergic disorders (Okada et al., 2010).

In an early report, the prevalence of AD in the canine population was estimated to be 15% (Chamberlain, 1974). More recently, estimates of 3-15% (Reedy et al., 1997) and 10% (Scott et al., 2001) have been stated. However, none of these figures are based on reliable epidemiological data and the true prevalence and incidence of AD in the dog population still remains unknown. The true prevalence of canine AD is difficult to determine because (1) mild cases are often successfully managed with symptomatic therapy without a diagnosis being made; (2) some clinical manifestations of AD may not be recognized by owners or veterinarians as part of AD, such as, chronic otitis, bacterial and *Malassezia* infections; and (3) there are no documented reliable methods to demonstrate that clinical disease is induced by allergen exposure in dogs with allergen hypersensitivity (Hillier & Griffin, 2001).

Further factors that may contribute to an increase in the incidence of canine AD are that dogs are spending more time indoors thus increasing exposure to common indoor allergens; there is a more wide-spread vaccination of puppies which may increase IgE antibody production (Frick & Brooks, 1983); and the practice of internal and external parasite control by dog owners is more common.

Therefore, at this time, there is insufficient data to speculate on the prevalence or incidence of AD in the general dog population (Hillier & Griffin, 2001).

4. Clinical Manifestations

4.1 Clinical Manifestations of Atopic Dermatitis in Humans

AD in human beings is most consistently characterized by the presence of pruritis. The clinical findings helpful in establishing a diagnosis of typical AD are (1) an onset under 2 years of age; (2) the presence of flexural dermatitis; (3) historical findings of a pruritic skin condition; (4) flexural involvement; (5) dry skin; and (6) asthma. (Williams et al., 1994). The most common distribution pattern of inflammatory skin lesions of AD includes the scalp, face, neck, and flexural surfaces of the extremities. Early lesions usually consist of dry skin and erythema, complicated by self-induced excoriations. Lichenification develops with chronicity secondary to chronic scratching. Secondary infections, especially with *Staphylococcus aureus*, are common (Beltrani, 1999; Griffin & DeBoer, 2001; Bieber, 2008).

4.2. Clinical Manifestations of Atopic Dermatitis in Dogs

Most atopic dogs begin manifesting signs between 6 months and 3 years of age. AD is not frequent to develop in dogs over seven years of age, however it has been reported in individuals as old as 12. Gender predisposition is still unknown, with different studies showing opposite tendencies. Breed predisposition is seen in canine AD, however there are probably variations between several regions and the popularity of the breeds. There are some studies from various time periods and geographic locations, that report the following breeds to be at a higher risk: Beauceron, Boston Terrier, Boxer, Bull Terrier, Bichon Frise, Cairn Terrier, Chinese Shar-Pei, Cocker Spaniel, Dalmatian, English Bulldog, English Setter, English Springer Spaniel, Fox Terrier, German Shepherd Dog, Golden Retriever, Irish Setter, Labrador Retriever, Lhasa Apso, Miniature Schnauzers, Pug, Scottish Terrier, Sealyham, Setters, Tibetan Terrier, Wire Fox Terrier, West Highland White Terrier and Yorkshire Terrier (Griffin & DeBoer, 2001; Griffin, 2008; Jaeger et al., 2009; Favrot, 2009).

Signs might be seasonal or non-seasonal, with or without seasonal exacerbation, depending on the allergens involved and other environmental conditions that influence AD known as flare factors (Dahl, 1990; Griffin & DeBoer, 2001; Griffin, 2008).

Dogs with AD generally have a history of pruritus in the face, ears, paws, extremities and/or abdomen, with or without recurrent skin or ear infections. These dogs can exhibit primary lesions, such as an erythematous macular to plaque-like eruption or an erythematous papular eruption. However, the consensus seems to be that some dogs with AD do not have visible primary lesions, even in pruritic areas, and that primary lesions consist only in erythema. Pruritus with no lesions is actually more common in AD. Secondary lesions, normally reflect chronic pruritis and trauma, chronic inflammation, concurrent secondary infections and bacterial overgrowth. These lesions include red-brown “salivary” staining, excoriations, self-induced alopecia, dry lusterless hair, hyperpigmentation, scaling and lichenification, in the pruritic areas, as the face (muzzle, periocular skin), concave ears, paws (dorsal and ventral), carpus and tarsi, flexural joints of the extremities, axillae, abdomen, groin, perineum, ventral tail and medial thighs (Griffin & DeBoer, 2001; Griffin, 2008; Olivry, DeBoer, Favrot, Jackson, Mueller, Nuttall & Prélaud, 2010; Favrot, 2009).

Atopic otitis externa or aural pruritus is also very common in canine AD (Griffin & DeBoer, 2001; Griffin, 2008). A history of lacrimation, ocular congestion or sneezing/rhinorrhea can be indicative of concurrent atopic conjunctivitis and rhinitis. Periocular and perinasal lesions may reflect co-existing pruritic atopic conjunctivitis and rhinitis, respectively (Favrot, 2009; Olivry et al., 2010).

“Allergic conjunctivitis” is a condition that refers to various hypersensitivity disorders that affect the eyelid, conjunctiva and/or cornea. In humans, it is the most frequent manifestation of eye allergy.

Allergic conjunctivitis has been diagnosed in dogs with CAD, however it is possible that this diagnosis is underreported or under valuated by clinicians, as seems to be the case in humans with AD (Lourenço-Martins et al., 2011).

In one study by Lourenço-Martins et al. (2011), the prevalence of ocular signs in a population of dogs with CAD has been evaluated. The ocular clinical signs most commonly seen were conjunctival hyperemia (90%), pruritus (73%) and chemosis (70%). Other, less frequent, signs included ocular discharge (60%), epiphora (57%) and corneal involvement (10%).

Secondary microbial infections most commonly caused by *Staphylococcus pseudintermedius* and *Malassezia* sp. yeasts, are also very common. An odor can result from these infections, which may be recognized by the owner before seeing any lesions. The lesions of pyoderma often include papules and crusted papules (most common), pustules, superficial erythematous rings with crusts, acute moist dermatitis, acral pruritic nodules, nodules and crusted lichenified plaques. These lesions are more often seen in the axillae, groin, and ventral neck folds, though they can occur in any location. *Malassezia* yeasts are most commonly associated with otitis externa. These cases present a light to dark brown waxy, moist exudate and the majority are associated with *Staphylococcus* bacteria. Cutaneous lesions are generally localized. Early lesions can appear normal or slightly erythematous. Chronic lesions include alopecia, hyperpigmentation, more intense erythema, greasy to yellow or gray brown crusty surface, plaque or lichenified plaque, reddish staining of hair or claws (Griffin, 2008; Favrot, 2009).

The age of onset, the presence of lesions in pruritic areas and the distribution patterns of the infection, rely on the owner characterization. It is important that the owner makes fact-based observations, and often this is only possible after the owner has been trained to correctly observe the clinical signs. Other important factor that must be determined is the actual behavior of the dog, instead of the owner's interpretation of what is normal or abnormal behavior (Griffin, 2008).

5. Flare factors

Ectoparasites

Fleas may complicate AD. Atopic dogs can also contract *Sarcoptes*. The concurrent presence of Demodicosis may be associated with immunosuppression, especially iatrogenic hyperadrenocorticism (Nuttall, 2008).

Microbial infections

Secondary infections must be identified and treated. Topical therapy on its own may be effective in the reduction of microbial populations and recurrence of infections. Immunosuppression may result in infections, but control of inflammation often reduces

colonization and infection with *Malassezia* and *Staphylococcus*. Dogs that are predisposed to develop pyoderma may benefit from long term systemic antibiotic therapy (Nuttall, 2008).

Stress

It has been proved that stress can exacerbate human inflammatory dermatoses, and this may also be true in animals. There is controversial evidence that behavioral therapy and pheromones may be helpful (Nuttall, 2008).

Environmental effects

High temperature and humidity, irritant surfaces or cleaning solutions may worsen skin diseases (Nuttall, 2008).

6. Diagnosis of atopic dermatitis

6.1. Clinical diagnosis

In atopic dermatitis there are no pathognomic clinical signs that permit a definitive diagnosis (DeBoer & Hillier, 2001). Rather, the diagnosis relies primarily on the clinical signs of the patient, history of the disease and exclusion of other pruritic diseases (Olivry et al., 2010). The clinical signs include severe pruritus and diffuse erythema affecting the ears, muzzle, eyes, flexor surfaces, feet and ventral body. Recurrent *Malassezia* and bacterial infections are common. Chronic lesions include alopecia, lichenification and hyperpigmentation, as seen above (Nuttall, 2008; DeBoer & Hillier, 2001). In the history of the disease usually there is a chronic, relapsing, normally steroid responsive dermatitis, history of other allergic diseases and a group of typical findings upon dermatological examination. These findings have been arranged into lists of clinical diagnostic criteria that have a strong correlation with AD (Willemse, 1986; Prélaud, 1998; DeBoer & Hillier, 2001; Nuttall, 2008; Favrot, Steffan, Seewald & Picco, 2010).

In 1986, Ton Willemse realized the similarity of canine “atopic skin disease” with some of the diagnostic features of human AD, and directly transposed the human criteria to the canine disease. However, these diagnostic criteria were never validated for sensitivity nor specificity (Willemse, 1986; Favrot et al., 2010).

In 1998, Pascal Prélaud and his colleagues reproduced the methodology of the UK Working Party and adapted it to the diagnosis of canine AD. Of five criteria, the combination of any three criteria had a sensitivity of 79% and a specificity of 81% for the diagnosis of canine AD (Prélaud, 1998; Favrot et al., 2010).

In 2009, in a study performed by Claude Favrot and colleagues using a large population of dogs, it was concluded that the use of a combination of five criteria had a sensitivity of 85%

and specificity of 79% to differentiate dogs with AD and those with other dermatoses. However, adding a sixth fulfilled parameter increased the specificity to 89%, but decreased the sensitivity to 58% (Table 1). In this study, it was also tested the sensitivity/specificity of Willemse's and Prelaud's criteria, which were 49/80% and 74/68% respectively. Favrot's criteria could be used in clinical practice as an aid to diagnosis, though these criteria are not absolute. However, ruling-out other differential diagnosis is expected to widely increase the specificity of the diagnosis (Favrot et al., 2010; Olivry, 2010). It is important to keep in mind that, in early stages of AD, lesions are unlikely to be seen at all characteristic sites, and pruritus might be present without observable lesions.

Table 1. The 2009 Favrot Diagnostic Criteria for Canine Atopic Dermatitis

1. Onset of signs under 3 years of age
2. Dog living mostly indoors
3. Glucocorticoid-responsive pruritus
4. Pruritus sine materia at onset (i.e. alesional pruritus)
5. Affected front feet
6. Affected ear pinnae
7. Nonaffected ear margins
8. Nonaffected dorso-lumbar area

A combination of five satisfied criteria has a sensitivity of 85% and a specificity of 79% to differentiate dogs with AD from dogs with chronic or recurrent pruritus without AD. Adding a sixth fulfilled parameter increases the specificity to 89% but decreases the sensitivity to 58%.

Source: Favrot C, Steffan J, Seewald W *et al.* A prospective study on the clinical features of chronic canine atopic dermatitis and its diagnosis. Veterinary Dermatology 2010; 21: 23–30.

It is critically important to recognize that other dermatoses can mimic AD. The major differential diagnosis of canine AD include: pyoderma, scabies, flea bite hypersensitivity, food sensitivity, *Malassezia pachydermatis* dermatitis, disorders of keratinisation and demodicosis. Such diseases must be ruled-out or controlled before the diagnosis of AD is made (Nagata, 2000; Nuttall, 2008; Olivry, 2010).

Pyoderma, most frequently caused by *Staphylococcus pseudintermedius* (formerly called *intermedius*), should always be considered as a secondary clinical manifestation of other underlying disorders, like hypersensitivity, ectoparasite infection, endocrinopathy or a defect in keratinization. The most common lesions are papules, pustules, erythema, epidermal collarets and focal alopecia. Although pyoderma can be found on any part of the body, it is more commonly present on the ventral abdomen and proximal limbs in dogs with AD (Nagata, 2000; Moriello, 2006; Favrot, 2009; Lloyd, 2009; Olivry et al., 2010; Thomas, n.d.)

Scabies is usually characterized by severe, papular, non-seasonal, steroid-refractory pruritus, accompanied by marked self-excoriation. Dogs may also exhibit peripheral lymphadenopathy and a peripheral eosinophilia. The diagnosis of scabies can be difficult, due to the difficulty of the recovery of mites in skin scrapings. Also, it is believed that the scabies allergen cross-reacts with that of the house dust mite *Dermatophagoides farinae*. Scabies is highly contagious to other dogs and 40% of owners will develop lesions. The diagnosis for scabies can be made by testing therapy or documenting an elevated titre to scabies antigen in the serum (Nagata, 2000; Nuttall, 2008; Favrot, 2009; Olivry et al., 2010; Thomas, n.d.).

Skin lesions associated with fleas can reflect a focal reaction at the bite site. Flea bite sensitivity is an allergic reaction to flea saliva. The most often affected regions are the dorsal lumbosacral region of the back, the base of the tail, the caudal and medial thighs and the inguinal region. The diagnosis of flea byte sensitivity is made by noting compatible clinical signs and the presence of fleas or flea feces, and confirmed by noting a good response to flea control. Dogs with AD are predisposed to the development of flea allergy (Nagata, 2000; Moriello, 2006; Nuttall, 2008; Halliwell, 2009).

The most common clinical signs of food intolerance is non-seasonal pruritus, with or without concurrent gastrointestinal signs, such as, flatus, borborygmus, or subtle bowel changes. Dogs with food intolerance may not respond to glucocorticoid therapy. Definitive diagnosis depends only on a good-to-excellent response to a novel diet in 6-8 weeks, and subsequently observing deterioration of the clinical signs in response to challenge and provocation studies (Nagata, 2000; Moriello, 2006; Nuttall, 2008; Favrot, 2009; Olivry et al., 2010) .

Malassezia pachydermatis, a lipophilic yeast, belongs to the normal cutaneous flora. *Malassezia* dermatitis is an inflammatory disease caused by the proliferation of this microorganism in the skin of dogs. Clinical signs include severe, erythematous, scaling, greasy, steroid-refractory pruritus. The diagnosis can be made by cytological techniques, such as tape striping, but the definitive diagnosis is only possible by response to therapy. Topical shampoos containing antimycotic agents, such as miconazole or ketoconazole, are effective treatments (Nagata, 2000; Moriello, 2006; Nuttall, 2008; Favrot, 2009; Lloyd, 2009; Olivry et al., 2010; Thomas, n.d.).

Defects in keratinization can be primary or secondary. The primary disorder can reflect a congenital error in the control of the process in keratinisation, also termed primary seborrhea. However, the most common defect in keratinisation is secondary and reflects an underlying disorder, such as allergy, endocrinopathy, or bacteria, yeast or ectoparasite infections. The primary disorder can be seen in certain breeds, like American Cocker Spaniel, English Springer Spaniel, West Highland White Terrier, Doberman Pinscher and Shih Tzu. The clinical spectrum is variable and characterized by greasy skin and hair (primary seborrhea),

or an accumulation on the skin of dry scales. Secondary infections will complicate these changes (Nagata, 2000).

Demodicosis is a parasitic disease characterized by the presence of excessive numbers of demodectic mites. It is not a contagious disease because *Demodex* mites normally inhabit the hair follicles and sebaceous glands. This disease is most commonly found in dogs under 1 year of age. The focal form occurs as round patches of alopecia with a normal or slightly scaling surface on the face and/or distal extremities. The localized form causes more diffuse hypotrichosis with slight scaling erythema and may be associated with pruritus. The generalized form includes a chronic dermatitis with diffuse alopecia, edematous erythema, scaling, comedones, irregular pigmented maculae, crust formation with exudates, and/or cellulites. The clinical diagnosis of demodicosis is made with hair examinations and skin scrapings. However, skin biopsy is the most reliable tool for the definitive diagnosis of the disease (Nagata, 2000; Nuttall, 2008; Favrot, 2009; Olivry et al., 2010; Thomas, n.d.).

The use of allergen-specific IgE serological or intradermal tests cannot be used for the initial diagnosis of AD in dogs. Many normal and atopic dogs exhibit positive reactions with either test, thereby markedly decreasing the test's specificity for the diagnosis of AD. Using a serologic test or intradermal test as a primary criterion for diagnosis of AD will, therefore, lead to misdiagnosis. However, such tests can be used for the following reasons:

1- Document whether or not the disease is associated with allergen-specific IgE (determining whether the dog suffers from AD or ALD);
2- Implement allergen-avoidance interventions (house dust mite elimination procedures);
3- Select allergens to be included in immunotherapy preparations

(DeBoer & Hillier, 2001; Olivry et al., 2010).

6.2. Allergy tests

After clinical diagnosis of canine AD, methods to support the diagnosis and identify to which allergens the atopic dog is hypersensitive can be made, commonly known as "allergy tests". These tests are essential if the clinician wants to perform allergen-specific immunotherapy, as a treatment. Currently there are two methods of allergy testing: *in vivo* methods, such as, intradermal or prick testing, and serum-based *in vitro* methods, total serum IgE quantification or measurement of allergen-specific IgE. Of these two *in vitro* tests, only allergen-specific IgE measurement is commercially available for the diagnosis of canine AD (DeBoer & Hillier, 2001).

6.2.1. Skin Prick Tests (SPT)

6.2.1.1. SPT in veterinary medicine

That the author is aware of, currently, there are no studies of SPT in dogs, and there is only one study of SPT in horses (Tilley, Sales Luís & Ferreira, 2010). In this study, SPT were performed in horses with equine recurrent airway obstruction (RAO) in which was suspected that they were sensitive to common aeroallergens present in the environment. For this study, several aeroallergens were used with the concentrations available commercially for use in human medicine. The results showed that all horses with RAO had several positive SPT results.

Even though, some authors still defend the use of IDT in these animals, they may "induce false positive reactions in clinically healthy horses, and require a more specialized technique and interpretation of results" (Tilley et al., 2010). Other advantages of SPT in comparison with IDT, shown in horses, are that the results are available immediately, have lower costs and the horses have little discomfort with this technique. However, if there is not a large healthy skin area due to extensive skin diseases this may be a limitation for the use of SPT. Some other contraindications include risk of anaphylaxis, although lower than with IDT, and the need of a trained clinician.

Therefore, this study has proven that with SPT, it was possible to identify possible triggers of allergy and clinical improvement in horses with RAO, after the implementation of appropriate aeroallergen evasion measures (Tilley et al., 2010).

In conclusion, SPT may be a valuable tool in veterinary medicine, for the determination of the allergens to which animals are sensitive, with the objective of recommending allergen avoidance measures and, eventually, perform allergen-specific immunotherapy.

6.2.1.2. SPT in human medicine

Prick-puncture tests are recommended as the primary test for the diagnosis of IgE-mediated allergic diseases in human medicine (Hillier & DeBoer, 2001).

It was Sir Thomas Lewis who, in 1924, first applied skin prick tests (Antunes, Borrego, Romeira & Pinto, 2009).

The advantages of the prick-puncture test versus intradermal skin test in humans, include: simplicity, rapidity, ease of interpretation, less discomfort, higher specificity, less irritation, and safety (low risk for severe adverse reactions). The disadvantages of this procedure are lower reproducibility and lower sensitivity compared to intradermal tests (Demoly et al., 1998; Hillier & DeBoer, 2001).

It is important that the clinician understands the clinical indications, correct technique, interpretation criteria, the risks and the limitations of SPT. Skin testing should always be an adjunct to history and physical examination and not a substitute for medical evaluation (Antunes et al., 2009).

SPT confirm the diagnosis of immediate hypersensitivity reactions. It can be used to select eviction measures and/or specific immunotherapy (Dreborg, 1989; Hamilton & Adkinson, 2003; Bernstein et al., 2008; Antunes et al., 2009).

The simplicity, rapidity of performance, low cost and high sensitivity make skin testing preferable to *in vitro* testing for determining the presence of specific IgE antibodies (sIgE). However, positive results must be correlated with history and physical findings since positive reactions don't necessarily imply diagnosis of allergy (Jacinto et al., 1992; Antunes et al., 2009).

The goal for the allergist is to perform skin testing with devices which minimize both false positive and false negative results while reducing patient discomfort. SPT should be a non-traumatic procedure and several sharp instruments such as a hypodermic needle, solid bore needle, lancet with or without bifurcated tip, and multiple-head devices, may be used (Carr, Martin, Howard, Cox & Borish, 2005; Antunes et al., 2009).

Multiheaded devices are designed to first be dipped into the extract bottles, then applied to the skin in one step. However, they appear to be more painful than single devices (Carr et al., 2005; Antunes et al., 2009).

Lancets should be sterilized, a fresh lancet for each prick, with normalized measures, and each lancet should be used only once for each extract, in order to avoid unintentional pricks, blood borne infections and allergen contamination.

Metal lancets with 1mm penetration limit are considered equally efficient and less painful than other synthetic devices with 1.4 or 1.6 mm penetration limits. The penetration limit is therefore a determinant factor when considering test efficacy and patient comfort, rendering metal lancets preferable when compared to other synthetic devices (Østerballe & Weeke, 1979; Almeida, Pires, Prates, Santa Marta & Pinto, 1996; Antunes et al., 2009).

Antiseptic solutions are recommended before SPT and skin must be dry before procedure.

Recommendations have been made regarding the appropriate placement of allergen extracts. The recommended distance for skin prick testing has varied between 2 and 5 cm (Voorhorst, 1980; Antunes et al., 2009).

For an accurate interpretation of wheal and flare reactions to allergens, both positive and negative tests should be used. As negative control, a saline solution, phenol at 0.5% or glycerine at 50% are recommended. For positive control, histamine dihydrochloride 10mg/ml or codeine phosphate at 9% can be recommended. Some authors advocate the use of histamine at 1 mg/ml (Scandinavian Society of Allergology, 1974); however, in a study by Morais de Almeida and collaborators (1996), this concentration consistently presented

negative results. Therefore, histamine at 1 mg/ml should definitely be abandoned (Malling, 1984; Almeida et al., 1996; Bernstein et al., 2008; Antunes et al., 2009).

The SPT result is considered positive if the wheal mean diameter is ≥ 3 mm. However, these results can only be validated with valid positive and negative control reactions. Histamine's reaction mean diameter has to be greater than 3 mm and the negative control should not exceed 3 mm with erythema diameter inferior to 10 mm () . However, papule size also depends on allergen concentration and number of allergens tested for which the patient is sensitized (Adinoff et al., 1989; Dreborg, 1989; Dreborg & Frew, 1993; Oppenheimer & Nelson, 2006; Antunes et al., 2009).

It is important to determine which allergens to test. This can be done according to the history of the patient, but also it should be taken into account the flowering season, types and levels of pollens and spores along the year and peak days of pollination, air composition and concurrent allergy symptoms during recurrent seasons to determine the appropriate outdoor aeroallergens for skin testing (Oppenheimer & Nelson, 2006; Bernstein et al., 2008; Antunes et al., 2009).

The presence of active cutaneous lesions constitutes a contra-indication to skin test procedures, due to the fact that it impairs the proper reading of SPT reactions. False-positive reactions can happen because of skin trauma, but also due to contaminated allergen extracts or cross-reactivity. Cross-reactivity depends on the structural and sequential similarity of the allergens involved, as between house dust mites, epidermal, but most of all, pollens (Aas, Backman, Belin & Weeke, 1978; Aas, 1980; Dreborg, 1989; Ferreira, Hawranek, Gruber, Wopfner & Mari, 2004; Antunes et al., 2009).

False-negative results can be due to the patient's age, concomitant drugs, extract quality/concentration and incorrect technique. It is important to also consider that non-IgE mechanisms, impossible to assess by SPT, may be involved (atopic-like dermatitis). The reduction in skin reactivity after specific immunotherapy can be found in sensitized patients. Concurrent drugs, such as antihistamines, anti-H_2 drugs, tricyclic antidepressants and topical corticosteroids can affect the results of skin testing, and so it is recommended a one-week drug-free interval before skin testing. Systemic corticosteroids, usually, do not affect skin reactivity when used for short periods of time (3-5 days). However, when used for long term therapy, false-negative results can be obtained. Topical corticosteroids should be discontinued 2 to 3 weeks prior to skin testing as prolonged use (over 3 weeks) may suppress wheal reaction (Pipkorn, Hammarlund & Enerbach, 1989; Des Roches et al., 1996; Pichler, Helbling & Pichler, 2001; Powe & Jones, 2006; Bernstein et al., 2008; Antunes et al., 2009).

These considerations are validated for human medicine. The objective of this study is to evaluate if these considerations can be extrapolated to canine AD and if SPT can be used as an alternative to IDT.

6.2.2. Intradermal tests (IDT)

Intradermal testing has been used for decades in both human and veterinary medicine, however, nowadays, it is less frequently performed in humans than skin prick testing. This is the *in vivo* method almost exclusively used in veterinary clinical practice (Hillier & DeBoer, 2001).

When IDT are performed, injected allergens attach to IgE antibodies bound to mast cells resulting in mast cell degranulation, and a wheal and flare reaction occurs. Therefore, IDT allows the determination of allergens responsible for an allergic condition and the selection of allergens for avoidance measures or allergen-specific immunotherapy (Hillier & DeBoer, 2001).

The selection and number of allergens to use for IDT will vary according to the patient's regional location. Most IDT panels include a selection of allergen extracts belonging to these groups: tree, grass and weed pollens, molds, house dust mites, insects and epithelia (Reedy et al., 1997; Hillier & DeBoer, 2001).

Use of allergen mixes is controversial, because there is the possibility of cross-reactivity among allergen extracts. Thus, it is recommended for IDT in dogs, the use of extracts containing individual allergens (Rosenbaum, Esch & Schwartzman, 1996; Platts-Mills, 1998; Hillier, Kwochka & Riester, 2000; Hillier & DeBoer, 2001).

Intradermal testing for the diagnosis of adverse food reactions is not recommended (Jeffers, Schanley & Meyer, 1991; Kunkle & Horner, 1992; Hillier & DeBoer, 2001).

In veterinary medicine, there are no standardized extracts. Extracts are only crudely standardized on weight/volume (w/v) or protein nitrogen units/milliliter (PNU/ml), however these methods are poor indicators of allergen content (Baer, Godfrey, Maloney, Norman & Lichtenstein, 1970; Hillier & DeBoer, 2001).

Allergens are commercially available in concentrations specified in units to indicate the potency of each allergen. The potency of the allergens may be affected by manufacture, storage conditions (such as temperature and duration of storage), accuracy of dilution of allergens for testing and contamination of allergens (Hillier & DeBoer, 2001).

The concentrations recommended for IDT in animals are 1000 PNU/ml for pollens, moulds and insects (Ackerman, 1988; Willis, Kunkle, Esch, Grier & Kubilis, 1996; Reedy et al., 1997; Scott et al., 2001); 1: 50 000 to 1: 1000 w/v or 1000 PNU/ml for house dust mites (Willis et al., 1996; Hillier et al., 2000; Hillier & DeBoer, 2001).

Therefore, these allergen extracts have to be diluted before testing due to the fact that they are commercially available in higher concentrations than the recommended.

There are many drugs that may adversely affect IDT:

- Antihistamines: The only antihistamine that seems to affect the skin test reactivity is hydroxyzine. However, a antihistamine withdrawal period of a minimum of 10 days prior to IDT, is recommended (Barbet & Halliwell, 1989; Hillier & DeBoer, 2001);
- Tricyclic antidepressant: Oral doxepin is known to suppress histamine reactivity and topical doxepin affects skin reactivity in human beings. These effects have not been studied on IDT reactivity in the dog (Sullivan, 1982; Karaz, Moeckli, Davis & Craig, 1995; Hillier & DeBoer, 2001)
- Glucocorticoids: Depend on the formulation and potency of the corticosteroid, dosage, frequency of administration, duration of treatment and individual patient factors. The recommendation for withdrawal of topical and oral glucocorticoids prior to IDT is 3 weeks minimum and for injectable glucocorticoids a minimum of 8 weeks (Reedy et al., 1997; Hillier & DeBoer, 2001; Scott et al., 2001)
- Other drugs: Progestational compounds, β_2-adrenergic agonists, bronchodilators and theophylline may also affect IDT reactivity (Malling, 1993; Demoly et al., 1998; Hillier & DeBoer, 2001; Scott et al., 2001).

Other factors that may affect IDT are:

- Season when IDT is performed, which seems to be only true for patients with seasonal disease. In these patients, the optimal time for IDT is at the end or within 2 months of the peak season (Hillier & DeBoer, 2001);
- Age of patient: very young dogs may still be developing a full range of allergen hypersensitivities and the mechanisms involved in wheal formation may not be fully functional (Hillier & DeBoer, 2001);
- Current or prior immunotherapy: the interpretation of IDT reactivity to allergens that constitute in current or past immunotherapy vaccines is impossible. Also, immunotherapy to some allergens may interfere with skin reactivity to other allergens during IDT (Hillier & DeBoer, 2001).

The skin should not be inflamed or infected at the time of IDT to allow interpretation of skin reactivity (Hillier & DeBoer, 2001).

Normally, dogs are sedated for better patient compliance and an easily performed testing. The sedatives and anesthetics that do not affect IDT are: xylazine hydrochloride, medetomidine, tiletamine/zolazepam, thiamylal, halothane, isoflurane and methoxyflurane. Sedatives and anesthetics that should not be used are: oxymorphone, ketamine/diazepam, acepromazine and propofol (Beale, Kunkle, Chalker & Cannon, 1990; Moriello & Eicker, 1991; Codner, Lessard & McGrath, 1992; Kennis, Robertson, Rosser & Hauptman, 1998; Vogelnest, Mueller & Dart, 2000; Hillier & DeBoer, 2001).

The most commonly used site for IDT is the skin of lateral thorax. This area is gently shaved and should not be scrubbed or washed. Then, skin test sites are marked and placed 3 cm apart (Reedy et al., 1997; Hillier & DeBoer, 2001; Scott et al., 2001).

Positive and negative control solutions are necessary to evaluate skin reactivity to allergen extracts. The positive control solution mostly used is histamine phosphate 0.001%. The negative control solution is usually 0.9% phosphate buffered saline (Reedy et al., 1997; Hillier & DeBoer, 2001; Scott et al., 2001).

Intradermal injections should be administered with tuberculin or 1.0 ml syringes with 26-27 gauge needle. The volume normally injected intradermally is 0.05 ml (Hillier & DeBoer, 2001).

Skin test reactions are typically read 15 minutes after injection. The criteria recommended for evaluation of the skin reactions are: (1) reactions at least equal to or greater than halfway between the reactions observed for the negative and positive controls, or (2) reactions at least 3 mm wider in diameter than the negative control (Reedy et al., 1997; Hillier & DeBoer, 2001; Scott et al., 2001).

Reactions are recorded with a score of 0, 1, 2, 3, or 4, where 0 is the same reaction as the negative control and 4 the same as the positive control. A reaction of 2 or greater is considered positive (Hillier & DeBoer, 2001).

False positive reactions are those seen at the IDT site that resemble the wheal and flare of a IgE-mediated reaction to allergen, but are not IgE-mediated. They can happen for many reasons, such as, irritant, contaminated or concentrated allergen extracts, incorrect technique, irritable skin and cross-reactions (dust mites with parasitic mites) (Reedy et al., 1997; Hillier & DeBoer, 2001).

False negative reactions may occur due to incorrect technique, low concentration of allergen, drug interference, host factors, incorrect selection of allergens and IDT performed too long after the peak season in patients with seasonal disease (Reedy et al., 1997; Hillier & DeBoer, 2001; Scott et al., 2001).

Some of the adverse reactions of IDT include: pruritus and inflammation at the injection site that can be treated with cold compresses, topical or short-acting oral glucocorticoids; local and generalized urticaria treated with systemic antihistamines and glucocorticoids; anaphylactic shock and collapse are rare, however require immediate and intensive treatment (Hillier & DeBoer, 2001; Scott et al., 2001).

Despite the common application of IDT in canine AD, as shown above, this test is not perfect. However, it is a valuable tool in the demonstration of allergen-specific hypersensitivity in the dog, and to choose which allergens to include in allergen-specific immunotherapy (Wood, Phipatanakul, Hamilton & Eggleston, 1999; Hillier & DeBoer, 2001; Baxter & Vogelnest, 2008).

6.2.3. Allergen-specific IgE measurement

Serum allergen-specific IgE assays are used to detect IgE antibodies directed against a panel of relevant allergens, according to the patient's history. Normally, these panels consist of pollen, mold, dust and epidermal allergens in various combinations. The patient serum is reacted with an individual allergen extract; then the antibodies that did not react are washed away, so that allergen-bound IgE is detected with a reagent specific for IgE. This IgE-specific reagent is coupled to an enzyme molecule [enzyme-linked immunosorbent assay (ELISA)] or radioisotope [radioallergosorbent test (RAST)]. The amount of bound IgE-specific reagent can be quantified by three different methods: colorimetric, fluorometric or radiometric. This amount is directly proportional to the amount of allergen-specific IgE.

The results of allergen-specific IgE serological tests can be affected by intrinsic patient or external environmental factors, such as age, seasonal factors, and prior immunotherapy. Unfortunately, this has been poorly studied in the dog, although these factors are known to influence this test in humans.

There is some evidence that suggests a lack of interference by recent therapy in allergen-specific IgE serological tests which is an advantage of the *in vitro* methods over intradermal testing, however more studies are necessary in this area.

Allergen-specific IgE serological tests are neither 100% sensitive nor specific, which means that some clinically healthy dogs have positive serum IgE tests (false positive) and some atopic dogs have negative serological tests (false negative). Some discrepancies between the results of intradermal testing and allergen-specific IgE serological tests done concurrently on the same patient can happen. The reasons for this are still unclear.

Concerning food allergen-specific IgE assays in dogs, there are some studies that found them to be insensitive, nonspecific and unreliable for the diagnosis of adverse food reactions (Jeffers et al., 1991; Mueller & Tsohalis, 1998; DeBoer &Hillier, 2001).

To summarize, these assays of allergen-specific IgE serology in canine atopic dermatitis are just tools to aid diagnosis and therapy, not as definitive diagnostic tests. Before their use, it is important to properly rule-out other possible diagnosis and a firm clinical diagnosis of atopic dermatitis is necessary (DeBoer & Hiller, 2001; Prélaud, 2008).

7. Treatment for canine AD

7.1. Etiological treatment

7.1.1. Allergen avoidance

There are some guidelines available that may help reduce the allergen load and exposure in patients hypersensitive to house dust mites and molds (Table 2). These guidelines can help owners decide how much time, effort and expense they want to invest in these avoidance measures. However, it is important to keep in mind that these measures are based on recommendations for human patients with allergies, and have not been evaluated in controlled studies in dogs (Hillier, 2002).

There is one study that tested the effects of benzyl benzoate, an acaricide, in the control of the environment of 60 house dust mite-sensitive dogs. In humans, house dust mite (HDM) elimination has been practiced for many years. Besides of the use of acaricides, other measures, such as, steam cleaning, ventilation and dehumidifiers may also be beneficial in the control of house dust mites allergens. Benzyl benzoate was chosen because of the ease of application, low cost, lack of damage to treated surfaces and lack of potential side-effects for dogs and owners. This study has shown that elimination of house dust mites and/or storage mites in the environment of dogs allergic to these allergens, seems to be a very effective tool in reducing clinical signs of house dust mite allergic dogs (Swinnen & Vroom, 2003).

Table 2. Allergen Avoidance and Control Measures

House dust mites
Cover mattresses, pillows, dog beds, chairs, and sofas with impermeable covers (vinyl).
Remove clutter, such as stuffed animals, from the pet's sleeping areas to prevent dust accumulation and to facilitate thorough cleaning.
Do not allow the pet into areas in which dust typically accumulates, such as closets, the laundry room, and under the beds.
Vacuum the house with a HEPA filter as frequently as possible—at least weekly.
Keep the pet outdoors during vacuuming and for one hour afterward.
Remove as many carpets and rugs as possible, especially from poorly ventilated rooms such as the basement, garage, and laundry room.
Wash linen, bedding, and blankets every week in hot water (> 130 F [54.4 C]).
Keep the humidity in the home at 30% to 45% relative humidity by using a dehumidifier, air conditioning, or a humidifier when needed.
Molds
Keep the dog away from freshly mowed grass, mulch, leaf piles, hay, and barns.
Keep the dog's kennel dry and clean.
Keep the humidity in the home at 30% to 45% relative humidity.
Keep the pet out of basements, closets, the laundry room, and bathrooms.
Keep the pet's bedding clean and dry.
Store food in a dry environment.
Clean moist areas where molds thrive with a fungicide or dilute sodium hypochlorite solution (1 part bleach to 9 parts water).

Source: Hillier, 2002.

7.1.2. Allergen-specific immunotherapy (ASIT)

Allergen-specific immunotherapy has been used for many years to treat dogs with AD. Multiple open studies and clinical observations suggest that ASIT is effective in controlling the clinical signs of dogs with AD (Griffin & Hillier, 2001).

ASIT consists of "administering gradually increasing quantities of an allergen extract to an allergic subject to ameliorate the symptoms associated with subsequent exposure to the causative allergen" (World Health Organization (WHO) definition – Bousquet et al., 1998), which means allergens are given in increasing doses up to a maintenance dose, or patient determined maximum dose.

There are many studies that have suggested the efficacy of aqueous ASIT for management of canine AD, and this intervention is considered one of the mainstays of treatment for this disease (Griffin & Hillier, 2001; Loewenstein & Mueller, 2009).

Allergen-specific immunotherapy should be used in dogs with a previous diagnosis of AD, in which skin testing or allergen-specific IgE serology enabled the identification of allergens that are likely to contribute to the disease and in which contact is hardly avoidable. Also, when anti-inflammatory treatment is not effective, or associated with unacceptable side effects, such as glucocorticoids, or is impractical to maintain for a long period of time, ASIT is

recommended, even in dogs with seasonal disease (Griffin & Hillier, 2001; Loewenstein & Mueller, 2009).

The mechanism of action of ASIT in dogs with AD is not as well studied as in human medicine. One study reported that after ASIT, peripheral blood mononuclear cells of dogs with AD stimulated with house dust mite antigen showed an increase in γ-IFN mRNA with no increase in IL-4 mRNA, suggesting a shift to a Th1 response by enhancing γ-IFN expression (Shida et al., 2004). A more recent study showed a significant increase in both Treg cell numbers and IL-10 concentrations after successful ASIT (Keppel et al., 2008). Some authors reported, similarly to the findings in humans, significant increases in allergen-specific IgG levels in dogs after 6 months of treatment (Hites et al., 1989; Fraser et al., 2004). Another study reported a loss of immediate-phase intradermal allergen test reactions that paralleled the clinical improvement in dogs receiving ASIT (Griffin & Hillier, 2001; Loewenstein & Mueller, 2009).

ASIT is believed to be the only therapy that can prevent the development of further allergies, and so giving the hope of a long-term remission, requiring a relatively low frequency of administration. Life threatening adverse reactions are rare and there are no reports of long-term side effects from ASIT in dogs. As awareness of AD by veterinarians increases, it is possible that the disease can be diagnosed in younger dogs at early stages of the disease development. Some advantages and disadvantages of ASIT are listed in Table 3 (Griffin & Hillier, 2001).

Table 3. Allergen immunotherapy: advantages and disadvantages

Advantages	Disadvantages
Less frequent treatment administration than symptomatic therapy	Syringes and needles dispensed to client
Less labor and time required, thus increased compliance	Owner fear of giving injections
No risk of long-term side effects reported	Risk of anaphylaxis
Low risk short-term side effects	Only available in glass vials, breakage risk and cost
Some dogs accept injections more readily than oral medications	Some dogs do not tolerate injections
May permanently alter the course of the disease with possible cure	Client education and support required for efficacy
Often more cost effective, especially in large breed dogs	Initially more expensive, with risk the expense will have no benefit
Preventative, not reactive treatment	
No monitoring tests required	

Source: Griffin & Hillier, 2001.

Clinical response to ASIT in dogs seems to be allergen-specific. In one double-blind study, dogs treated with a nonspecific standard set of allergen exhibited a median improvement of clinical scores of 18%, while dogs treated with the allergens specifically selected based on

intradermal testing had a median improvement of clinical scores of 70% (Willemse, 1994; Griffin & Hillier, 2001; Loewenstein & Mueller, 2009).

So, it appears to be important to consider the clinical significance of positive reactions detected with intradermal testing or allergen-specific IgE serology, by evaluation of the clinical history and likely exposure to allergens in each patient, in order to have better clinical improvement (Griffin & Hillier, 2001; Loewenstein & Mueller, 2009).

As many dogs with AD have multiple allergen hypersensitivity, mixtures of allergens are often prescribed. However, some problems may occur with allergen mixtures, such as an excessive dilution, that may be responsible for suboptimal dosing and more rapid allergen deterioration; and a loss of allergenicity because of the enzymatic activity of some allergen extracts. An example is the effect of mold allergen extracts (high in proteases) on weed, tree and grass pollen extracts, that has been demonstrated to decrease significantly the biologic activity of grass and weed pollens when these allergens are mixed and stored together (Rosenbaum et al., 1996; Griffin & Hillier, 2001; Loewenstein & Mueller, 2009).

Subcutaneous injections are, currently, the standard route of administration for ASIT in dogs (Griffin & Hillier, 2001).

There is controversial data about the numbers of allergens that can be included in a single treatment set for ASIT. Dogs that show hypersensitivity to multiple allergens theoretically could be successfully managed with ASIT with only some of the allergens causing positive allergy test results, however more studies are needed to comprehend the true mechanism involved in ASIT (Griffin & Hillier, 2001; Loewenstein & Mueller, 2009).

In human medicine, the allergen dose for optimal clinical response has been defined for many standardized allergens. However, no standardized allergen extracts are available for use in veterinary medicine. Several textbooks recommend that maintenance vials with a total concentration of 10,000-20,000 PNU/ml of allergens should be used. It is important to keep in mind that allergen extracts in aqueous solution lose potency with time, and an even higher loss of potency is seen with increasing dilution. The potency of allergens stored for more than 2 months, which is often the case with allergen immunotherapy vials, should be further evaluated (Griffin & Hillier, 2001; Loewenstein & Mueller, 2009).

The interval between injections during initial loading is recommended to be 2-3 days up to 1 week as the dose of aqueous allergen is increased, and for maintenance protocols of between 5 and 20 days. According to one open study, a modified protocol based on the patients' response to the injected allergens could decrease interval between maintenance injections, with an average of 10 days, but ranging from 3 to 21 days. The optimal dosing interval for loading and maintenance allergen injections has not been established in controlled studies, and can be highly variable between individual patients. The adjustment of the injection frequency and dose of allergen extract to the patients' requirements is important

in optimizing the efficacy of the therapy (Rosser, 1998; Griffin & Hillier, 2001; Loewenstein & Mueller, 2009).

Several studies imply that ASIT is effective in the treatment of canine AD. These studies suggest that 50-100% of dogs receiving ASIT will have at least a 50% improvement in clinical signs after more than 4 months of therapy. It is believed that the efficacy of ASIT can be improved by selecting allergens based on the results of both allergen-specific serology and intradermal testing.

The time to maximal clinical benefit and total duration of ASIT are currently unknown in veterinary medicine, however these factors have great impact on the patients' well-being and on owners' compliance. Some studies reported that dogs receiving aqueous ASIT only had beneficial effects after 8-9 months of treatment.

The long-term efficacy of ASIT in canine AD has not been currently evaluated in a controlled study. The clinician should be aware that this treatment could be life-long in some dogs, but other patients can have complete remission of clinical signs.

The efficacy of ASIT in canine AD can be affected by several factors, such as the age at onset of disease, age at the time of beginning ASIT, duration of the disease, severity of clinical signs, breed, strength of intradermal test reactions, number of allergens used and type of allergen to which the patient is hypersensitive (Griffin & Hillier, 2001; Loewenstein & Mueller, 2009). However, one recent study showed that the success rate of ASIT was not significantly affected by the age of the dogs when the disease developed or when the treatment began, the type of allergens or the method used to identify the allergens (intradermal or serological) (Schnabl, Bettenay, Dow & Mueller, 2006).

The effects of concurrent therapy with glucocorticoids during ASIT have not been evaluated in the dog, but these interactions could be important because of the importance of T-lymphocytes in the efficacy of ASIT. Besides the effects of glucocorticoids on the immune response to ASIT, it is also possible that these drugs can mask a positive clinical benefit of ASIT, or a covert adverse reactions that may need a modification of the treatment protocol. Some authors (Scott et al., 2001) believe that ASIT could still be effective if oral prednisolone doses are as low as possible or administered on a alternate day basis, while others (Griffin et al., 1993) defend that glucocorticoids should be avoided in the induction phases of ASIT. Cyclosporine does not seem to inhibit intradermal reactivity in the dog when short-term treatment at 5 mg/kg daily. However, long term effects on ASIT are currently not known.

Active follow-up of patients is an important factor in the potential success of ASIT. The efficacy of ASIT in dogs could be improved by monitoring the response in the individual patients for several reasons, such as allergen extracts are poorly standardized in veterinary medicine and biological potency may be variable, dogs have variable size and weight, the time until clinical improvement can range from months to years, allergen immunotherapy can exacerbate the clinical signs and some dogs seem to present only transient improvement

following each injection, so it may be necessary to make alterations of the treatment protocol. Furthermore, dogs with AD may present significant concurrent skin disease (*Staphylococcus* and *Malassezia* infections), and these flare factors could be a reason, if not controlled, for the failure in response of ASIT.

Compliance by owners of dogs with AD seems to be low. The main reason for cessation of ASIT is lack of improvement. Therefore, it is important to maintain frequent communication with the owners and re-evaluation of the patients, in order to improve compliance with ASIT for a sufficient duration of time to assess possible clinical response. So, it is believed that patient monitoring is essential in the efficacy of ASIT (Griffin & Hillier, 2001; Loewenstein & Mueller, 2009).

Adverse reactions to ASIT are uncommon in dogs. These reactions can be divided into local and systemic. Small local reactions with swelling and/or erythema are relatively common, while pronounced local reactions with edema and pain or pruritus are, only, occasionally seen, and normally, do not require modifications in treatment protocol. Systemic reactions are reported to occur in about 1% of the dogs and may include weakness, depression, anxiety, sleepiness, panting, hyperactivity, diarrhea, vomiting, increased bowel sounds, frequent swallowing, urticaria/angioedema, pruritus, anaphylaxis and collapse. The most frequently seen adverse effect is a worsening of clinical signs and pruritus, immediately after an injection or 1 or 2 days later, that can persist for hours or even days. It has been recommended pretreatment with antihistamines in dogs, in order to prevent these reactions. The clinical deterioration with injections of allergen extracts can be indicative that the patient's maximum tolerance of allergens has been exceeded. Protocol modifications are necessary in these cases (Griffin, 1993; Griffin & Hillier, 2001; Loewenstein & Mueller, 2009).

7.2. Symptomatic treatment

7.2.1. Improving skin barrier function

There are some important nutrients that may help to improve the barrier function such as, zinc, long chain ω-3 essential fatty acids (EFAs), inositol, choline, histidine, pantothenate, nicotinamide, aloe vera and curcumin, and have the ability to decrease inflammation, alter eicosanoids, improve epidermal lipid barrier formation, up regulate fibroblasts, proteoglycan synthesis and TGF-β production (Nuttall, 2008).

Topical therapy also has benefits, like physical removal of allergens through bathing with moisturizing shampoos and conditioners which also prolong hydration, and may improve the skin lipid barrier. Other topical products that can be helpful are colloidal oatmeal, which has direct anti-pruritic action; linoleic acid, that improves the skin lipid barrier; vitamin E and mono-oligosaccharides reduce TNF-α production and prevent microbial adherence; and

piroctone olamine modulates the skin flora. Ear cleaners and anti-microbial or anti-scaling shampoos may also be helpful. It may be necessary to alternate between antimicrobial and emollient shampoos (Nuttall, 2008).

Supplementation with ω-3 EFA eicosapentaenoic acid (EPA) and ω-6 EFA gamma-linolenic acid (GLA), may result in altered plasma levels and incorporation into cell membranes, that can lead to reduced production of inflammatory leukotrienes and prostaglandins and improved skin lipid barrier. However, clinical trials have shown variable results, and no relationship between efficacy and ratio of ω-3/ω-6 EFAs was proven, although recent studies showed that high quality, EFA enriched diets can be beneficial in canine AD (Nuttall, 2008). In one study it has been proven that supplementation with EFA can lead to decreased glucocorticoid usage after 8 weeks, although it may cause minor digestive signs (Olivry, Foster, Mueller, McEwan, Chesney & Williams, 2010).

7.2.2. Systemic and topical calcineurin inhibitors

Cyclosporine A (CsA), as a systemic calcineurin inhibitor, has the effect to suppress T-cells and other inflammatory reactions, such as, mast cells and eosinophils. These affect antigen presentation, IgE production, mononuclear cell activity and the development of inflammatory lesions, although at the doses used in canine AD, cyclosporine is immune-modulating instead of immunosuppressive (Barata, 1999; Nuttall, 2008; Olivry et al.,2010).

Cyclosporine is rapidly absorbed and distributed with bioavailability that ranges between 15-60% in dogs and is not affected by food. This drug is metabolized via the P_{450} cytochrome system, and many drugs may affect this metabolism, such as, itraconazole and ketoconazole that can decrease the metabolism, increase plasma concentrations and the likelihood of adverse effects, and phenobarbital which increases the metabolism and decreases plasma levels (Barata, 1999; Nuttall, 2008; Olivry et al., 2010).

The dosage normally used for canine AD is 5 mg/kg once daily, and dose adjustments are made according to clinical response. It has been shown that cyclosporine at this dose is as effective as glucocorticoids (prednisolone and methylprednisolone), however this may take at least 2 weeks to become apparent. Glucocorticoids may be used concomitantly at first to accelerate remission (Nuttall, 2008; Olivry et al., 2010).

It is believed that cyclosporine has minimal effect in intradermal and serology testing, and does not affect the response to allergen-specific immunotherapy (ASIT) (Nuttall, 2008; Goldman, Rosser Jr., Petersen & Hauptman, 2010).

Normally, cyclosporine is well tolerated. The most common side effects include transient anorexia and vomiting, that can be eased by administering with food and/or by using the gastrointestinal protectant sucralfate or H_2 blocking agents like ranitidine, in case of persistent vomiting. Hirsutism, transient alopecia, gingival hyperplasia, papillomatosis,

diarrhea, lameness and muscle tremors, and erythema and edema of the ears are other uncommon adverse effects, however these are dose-dependent and reversible (Nuttall, 2008; Olivry et al., 2010).

Immunosuppression is a potential concern. Inhibition of cell-mediated immunity can result in bacterial and protozoa infections, dermatophytosis and demodicosis, however, in clinical practice, there seems to be a very low risk and most dogs with AD experience fewer secondary infections after treatment. Inhibition of T-cell function and β-cell activation may affect response to vaccination, so it is recommended to withdraw treatment for up to 2 weeks prior to and after vaccination, although this will lead to worsening of clinical signs (Nuttall, 2008).

The long-term safety of cyclosporine administered at this dosage to dogs with AD has not been established beyond 6 months (Olivry et al., 2010).

There is one double-blinded randomized controlled trial (RCT) that tested the hypothesis that oral administration of CsA, given at 5 mg/kg once daily for 6 weeks, led to a decrease in skin lesions and pruritus in dogs with non seasonal AD. During this trial it was noticed a rapid reduction in lesional and pruritus scores within the first 3 weeks of treatment with CsA. It was also observed that oral CsA provided “good-to-excellent” (> 50% decrease from baseline) reduction of pruritus in approximately 75% of patients, leading to the conclusion that this drug is one of the most potent anti-allergic drug available to treat dogs with AD. Also, changes in laboratory parameters, such as, increases in blood urea nitrogen and creatinine, observed in human patients given oral CsA for many weeks, or changes in complete blood counts and serum chemistry, were not seen commonly in dogs given CsA. This study provides evidence that CsA is highly effective in reducing clinical signs in canine AD, and that this efficacy is of similar potency to that of prednisolone. Therefore, it is believed that oral CsA should be considered an alternative to oral glucocorticoids in the management of canine AD (Barata, 1999; Olivry et al., 2002).

Tacrolimus, a topical calcineurin inhibitor, has a similar mechanism of action to cyclosporine. A 0.1% tacrolimus ointment, applied once or twice daily, has been shown, in one study, to lead to a greater that 50% improvement in atopic dogs with localized lesions. The magnitude of effect was higher when used twice daily. Plasma levels were low and the only side effect seen was minor self trauma immediately after application, which appears to be safe to use in the short-term. However, treatment of generalized skin lesions, besides of being impractical and costly, has lower benefit (Barata, 1999; Nuttall, 2008; Olivry et al., 2010).

7.2.3. Systemic and topical glucocorticoids

Glucocorticoids are the most commonly used drugs in veterinary dermatology. They are cheap, easy to use and highly efficacious, however associated with many side effects. They

act by inhibiting various molecules involved in immunity and inflammation, therefore resulting in immunosuppression and decreased inflammation (Barata, 1999; Nuttall, 2008).

These drugs are very effective in canine AD, but must be used with caution and as a last resort. Alternate medications may help reduce the dose and frequency of application. Seasonal AD, that needs treatment for 3-4 months each year, can be managed with minimal side effects. For treatment of flares of inflammation, short term management (0.5-1 mg/kg once daily for 3-5 days) may also be used (Nuttall, 2008).

Systemic glucocorticoids are needed in more severe or generalized lesions. Prednisolone at 0.5-1.0 mg/kg once daily is recommended until remission. Then gradual weaning is necessary to allow the hypothalamic-pituitary-adrenal (HPA) axis to recover. Injectable preparations are not recommended unless absolutely necessary, because they cannot be withdrawn, the dose cannot be altered, and the HPA axis is not allowed to recover (Barata, 1999; Nuttall, 2008; Olivry et al., 2010).

Glucocorticoids suppress reactions to intradermal and serologic allergen testing (although the effect seems to be less marked in serology), so it is recommended to withdraw topical glucocorticoids for two weeks, short acting oral glucocorticoids for three weeks and longer acting injectable glucocorticoids for six weeks prior to allergy testing. Dogs in long term therapy or with iatrogenic hyperadrenocorticism need longer withdrawal times. Administration of glucocorticoids to control inflammation in the induction phase of immunotherapy, does not seem to affect the response rate of ASIT (Nuttall, 2008).

Adverse side effects result from glucocorticoid and mineralocorticoid activity, suppression of HPA axis and endogenous steroid production. Common side effects include polyuria and polydipsia, which can be reduced by using methyl-prednisolone, polyphagia and weight gain, managed with a low calorie diet, panting and behavioral changes. In long term therapy, it is possible to occur immunosuppression and secondary infections. Demodicosis, dermatophytosis and infections with intracellular organisms may happen due to inhibition of cell-mediated immunity. Immunosuppression and alterations in skin barrier function can be responsible for superficial pyoderma. Production of dilute urine contributes to the development of cystitis. Side effects of these drugs are common, predictable and dependent on glucocorticoid doses and treatment duration (Nuttall, 2008; Olivry et al., 2010).

Short term treatment with glucocorticoids may be used, if cyclosporine has to be withdrawn, prior to vaccination, because humoral immunity is less affected and dogs can develop appropriate antibody titers following vaccination (Nuttall, 2008).

Topical glucocorticoids, such as 0,015% triamcinolone spray, are directed to affected skin and can avoid the use of systemic therapy. They are effective in the treatment of pruritus and relatively hairless skin, “hot-spots”, ears and eyes (Barata, 1999; Nuttall, 2008; Olivry et al., 2010).

Hydrocortisone aceponate is a topical diester glucocorticoid used for the treatment of pruritus in canine AD. Topical diester glucocorticoids are rapidly absorbed and have potent anti-inflammatory effects in the epidermis and dermis. Metabolism within the dermis ensures that almost none active compound reaches deeper tissues, minimizing systemic side effects and skin thinning. The topical formulation, small dose volume, small droplet size and volatile carrier ensure a quick and easy application. Two sprays from a distance of 10 cm penetrate the coat and treat an area of 10 x 10 cm (Nuttall, 2008).

Some unpublished studies demonstrate good efficacy and safety in short term therapy of “hot spots” and flea allergic dermatitis. Some studies found that Cortavance® is effective and well tolerated in canine AD. Once daily administration induces remission, after which a maintenance protocol of every other day therapy can be implemented (Nuttall, 2008).

7.2.4. Antihistamines

First generation antihistamines clemastine and a combination of chlorpheniramine and hydroxyzine, and the second generation antihistamine oxatomide, have been shown to have only medium efficacy in the management of canine AD. This may be due to inappropriate doses or frequencies of administration, as these parameters have been extrapolated from human pharmacological data, without further studies in dogs. However, they may have some synergistic effects when used together with EFAs and glucocorticoids. Adverse side effects of first generation drugs are uncommon and normally include drowsiness and sedation. Second generation antihistamines may be responsible for gastrointestinal tract disorders and cardiac arrhythmias (Nuttall, 2008; Olivry et al., 2010).

7.2.5. Other therapeutic options

Phosphodiesterase inhibitors are also immunomodulating. There is evidence of low to medium efficacy of pentoxifylline at 10 mg/kg 2-3 times daily and no side effects have been seen. Arofylline, at 1 mg/kg twice daily, has medium to high efficacy, however is responsible for unacceptable vomiting (Barata, 1999; Nuttall, 2008; Olivry et al., 2010).

Leukotriene inhibitors, such as 5-lipoxygenase inhibitors, have been proven to have low efficacy for the treatment of canine AD. However, side effects are uncommon (Barata, 1999; Olivry et al., 2010).

Misoprostol is a prostaglandin E1 analogue which inhibits late-phase inflammatory reactions by preventing activation of basophils, mast cells and eosinophils. There is evidence of medium efficacy of this drug in canine AD at 6-10 µg/kg and is well tolerated by most dogs (Nuttall, 2008; Olivry et al., 2010).

Antibacterial and antifungal medications can be helpful when concomitant skin infections are present or when microbial allergens are suspected to be involved in the perpetuation of canine AD (Olivry et al., 2010). For dogs with superficial pyoderma, 3 weeks of an appropriate antibiotic should be used. If the dogs manifest deep pyoderma, 4 to 8 weeks or longer may be necessary. If *Malassezia* dermatitis is present, topical therapy alone, or together with systemic antifungal therapy is recommended. A major reduction in pruritus is, normally, seen when secondary infections are controlled. Also, antipruritic therapies are, usually, more effective if the patient is free of these secondary infections (Thomas, n.d.).

III. Skin Prick testing in healthy non-atopic dogs

1. Materials and Methods

1.1. Objective of the study

This study is composed by three phases.

1. Determine whether or not the SPT are doable in dogs;
2. Verify if the available concentrations for the allergens used in human medicine do not induce irritant “false positive” reactions in healthy non-atopic dogs;
3. Confirm the correlation with the results of other methods of skin testing already established for CAD, intradermal tests, in order to see if the concentrations available for SPT are enough to induce clinically significant positive skin tests in atopic dogs.

In the present study, it has only been possible to perform the first two phases in more detail, due to unexpected delayed in the acquisition of allergenic extracts for IDT from Greer Laboratories. However it was possible to test four atopic dogs using both SPT and IDT.

1.2. Dogs

Twenty-two clinically healthy dogs, belonging to the kennel of the Teaching Faculty Hospital, were used in this study, after the consent of the Faculty of Veterinary Medicine, Technical University of Lisbon. Additionally, the consent of the Ethics Committee has also been given to perform this study.

Inclusion criteria:

1. Healthy;
2. Non-existent history, past or present, of pruritus or skin and ear diseases;
3. No abnormalities found on the general physical examination, dermatologic examination, and complementary tests if needed;

Exclusion criteria:

1. Presence of any disease;
2. Existent history of pruritus or skin and ear diseases;
3. Abnormal physical and/or dermatological examination;
4. Pregnant or lactating females;
5. Dogs under six months old.

The dogs included in our study were 14 intact males and 8 intact females. Their ages ranged from 1 to 14 years of age (mean age 7.5 years) and weights ranged from 10.5 to 37.2 kg (mean weight 23.85 kg). They were all mix-breed dogs.

All the non-atopic dogs included in our study had a normal general physical examination and dermatological examination.

As a preliminary study of the third phase of the project, four atopic dogs, diagnosed in the Dermatology consultations at the Teaching Hospital of the Faculty of Veterinary Medicine, Technical University of Lisbon, were also used for testing with both IDT and SPT. Therefore, a total of twenty-six dogs were used in this study.

1.3. Allergens

Fifteen aqueous allergens commercially available for use in human medicine, were used for SPT. The allergens included two house dust mites, two storage mites, five fungi, four grass pollens, one tree pollen and one weed pollen. All the allergens were obtained from ALK-Abelló, Portugal. These allergens were stored in glass vials at 4ºC. The allergens, as well as their respective concentrations, used in this study are described in Table 4.

Table 4. Allergens and respective concentrations

Allergen	Type of allergen	Concentration
Dermatophagoides pteronyssinus	House dust mite	30 HEP Der p 1: 40 µg/ml Der p 2: 20 µg/ml
Dermatophagoides farinae	House dust mite	30 HEP Der f 1: 40 µg/ml Der f 2: 20 µg/ml
Lepidoglyphus destructor	Storage mite	10 HEP Lep d 2: 30 µg/ml
Tyrophagus putrescentiae	Storage mite	10 HEP
Alternaria alternata	Fungi	30 HEP Alt a 1: 25 µg/ml
Aspergillus fumigatus	Fungi	1:20 w/v
***Cladosporium* mistura**	Fungi	1:20 w/v
Mucor mucedo	Fungi	1:20 w/v
Penicilium notatum	Fungi	1:20 w/v
Lolium perenne	Grass pollen	30 HEP Lol p 5: 60 µg/ml
Phleum pratense	Grass pollen	30 HEP Phl p 5: 60 µg/ml
Secale cereale	Grass pollen	30 HEP Sec c 5: 60 µg/ml
Dactylis glomerata	Grass pollen	30 HEP Dac g 5: 60 µg/ml
Olea europaea	Tree pollen	30 HEP Ole e 1: 180 µg/ml
Parietaria judaica	Weed pollen	30 HEP Par j 1: 20 µg/ml

1.4. Technique

1. Approximately a 20 x 10 cm area of hair from the lateral flank of the thoracic region was clipped. Whenever possible, this was done the day before to avoid some degree of irritation of the skin and subsequent hyperreactivity;
2. The distance between each test was 3 cm and test sites were marked with a pen;
3. Lancets were pressed 90º to the skin surface through a drop of extract or control solutions;
4. Sterilized lancets were used for each prick, with 1 mm penetration limit and each lancet was used only once for each extract;
5. A saline solution and histamine dihydrochloride 10 mg/ml as negative and positive controls, respectively, were used;
6. The skin reactions were measured immediately after the prick tests were applied and 15 minutes later;
7. The skin reaction was considered positive if the wheal's area was 7 mm^2 or higher, which corresponds approximately to a mean diameter of 3 mm;
8. The tests were considered valid when the histamine's papule (positive control) mean diameter was greater than 3 mm and negative control did not exceed 3 mm.

Specific ways to avoid pain or discomfort used in this study:

1. Time in hospital was reduced to the minimum necessary;
2. Dogs were closely monitored for pruritus or discomfort and treated accordingly if needed.

2. Results

In all twenty-two healthy non-atopic dogs included in our study, the results of SPT for the fifteen allergens at the concentrations standardized for human medicine, described above in Table 4, showed that 0 healthy dogs reacted to the maximum concentration available for each allergen (Table 5). The histamine positive control induced the formation of a papule with a mean diameter greater than 3 mm in all dogs, and the saline solution negative control didn't form a papule or formed a papule with a mean diameter of less than 3 mm, showing the validity of the test (Figures 1 and 2).

The four atopic showed the following results:

- 1/4 showed sensitivity to house dust mites (*D. pteronyssinus* and *D. farinae*) and storage mites (*T. putrescentiae* and *A. alternata*) using both IDT and SPT;
- 2/4 had positive SPT results to house dust mites and negative SPT results to storage mites with positive IDT results to both house dust and storage mites;
- 1/4 did not react to the histamine positive control, thus the test was not considered valid.

Figure 1. Papule induced by the histamine positive control (outline)

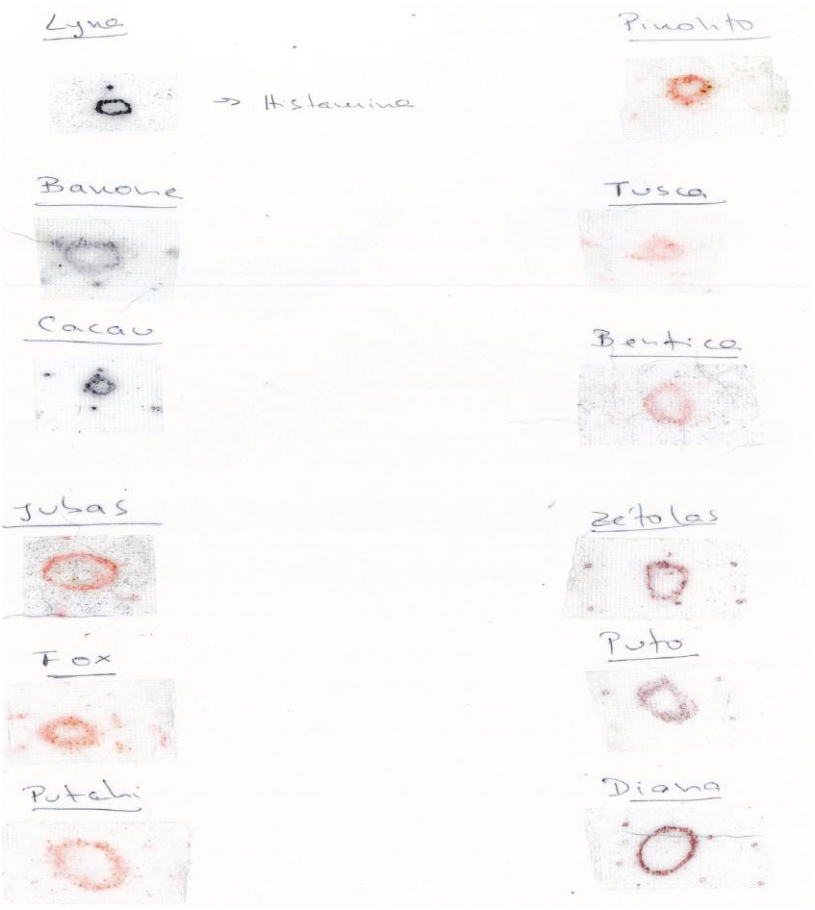

Figure 2. Papule induced by the histamine positive control (outline)

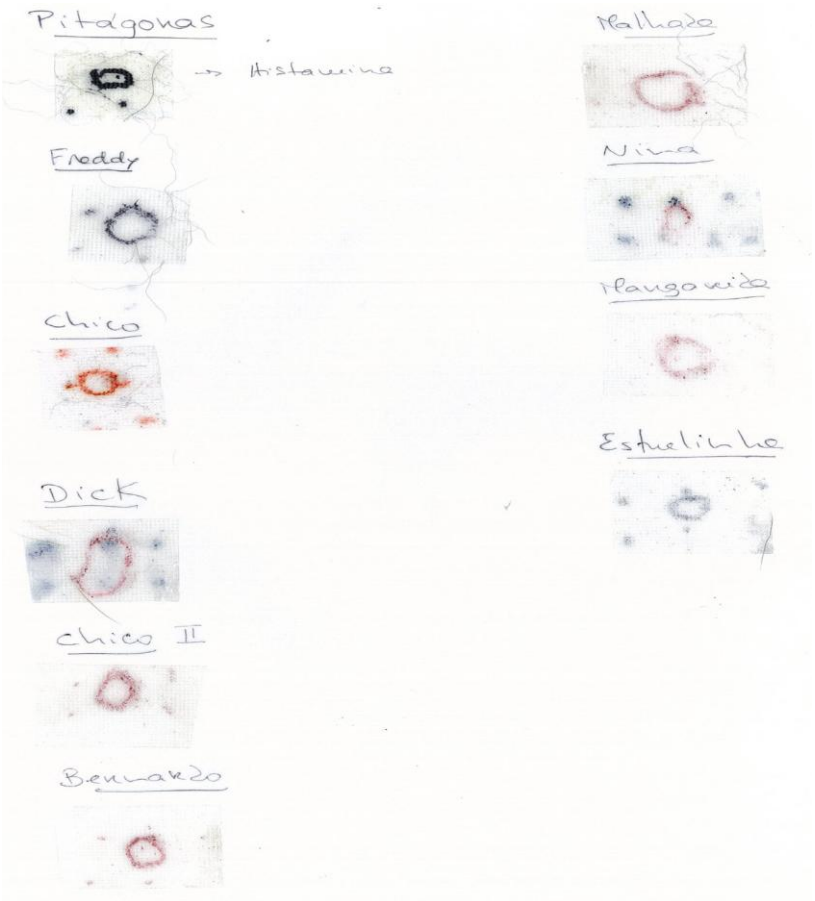

Figure 3. SPT results and papule outline induced by the histamine positive control of healthy non-atopic dogs

Figure 4. SPT results and papule outline induced by the histamine positive control of healthy non-atopic dogs

Table 5. Results of SPT in twenty-two healthy non-atopic dogs

Allergens \ Dogs	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Histamine control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Negative control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D. pteronyssinus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D. farinae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L. destructor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T. putrescentiae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. alternata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. fumigatus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. mistura	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M. mucedo	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. notatum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L. perenne	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. pratense	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. cereale	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D. glomerata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O. europaea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. judaica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(+) – positive skin test, wheal diameter above 3 mm
(-) – negative skin test, wheal diameter 3 mm or less

3. Discussion

Prick-tests are doable and easy to perform in dogs and should be further studied as valid alternative/complement to intradermal skin test techniques.

All the twenty-two healthy non-atopic dogs used in this study showed negative skin reactions to the allergens used (as showed in Table 5). As none of the dogs developed positive skin reactions at the highest concentrations available for each allergen, there was no need to test serial allergen dilutions. Also, all these dogs had corresponding skin reactions to the positive and negative control solutions, showing the test was valid.

It would be interesting, however, to be able to use higher concentrations, as the ones used could be weaker than needed for the dogs to show sensitization reactions. Due to practical reasons (commercial unavailability of other extracts concentrations), this was not done. However, even in a technique used for many years in dogs, such as IDT, it is still discussed the maximum concentration that does not induce false positive reactions in more than 10% of non-atopic dogs (irritant threshold concentrations) (Bauer, Hensel, Austel & Keys, 2010).

Four atopic dogs were tested for these allergens, as a preliminary study of a more extensive study, in this regard. These dogs mainly showed sensitivity to house dust (*D. farinae* and *D. pteronyssinus*) and storage mites, when using IDT. When using SPT, one of the four atopic dogs had a corresponding skin test result with IDT (Figure 5 and 6), however, two of the four atopic dogs showed sensitivity only to house dust mites (Figures 7 and 8). One of the four atopic dogs, although, did not react to the histamine positive control, therefore, the test was not valid. This means that only one dog, of the 26 dogs tested, did not have a valid SPT. However, this dog had a dark and thickened skin on the lateral thorax, so the test was repeated in the skin of the ventral abdomen, and in this area, there was a reaction to the histamine positive control (Figure 9). This could mean that a dark and/or thickened skin may be a limitation for the use of SPT, and that it may be necessary to perform SPT in another location where there is a more sensitive and thinner skin (such as, the ventral abdomen or axillae).

These preliminary results in atopic dogs may be explained by the fact that the standardized concentrations available for SPT in human medicine are not high enough to induce positive skin reactions to the allergens, which show positive reactions using intradermal testing, in this particular case this happened especially with storage mites. However, it is rare that dogs have positive reactions to storage mites without showing sensitivity to house dust mites, as well. It is possible that cross-reactivity factors may be present between these allergens, therefore, it should not be excluded that some of the positive reactions are due to a higher concentration (irritant) of these allergens (Lourenço-Martins, 2010).

Figure 5. Papule induced by *D. pteronyssinus*, *D. farinae*, *T. putrescentiae* and *A. alternata* allergens in one atopic dog (outline).

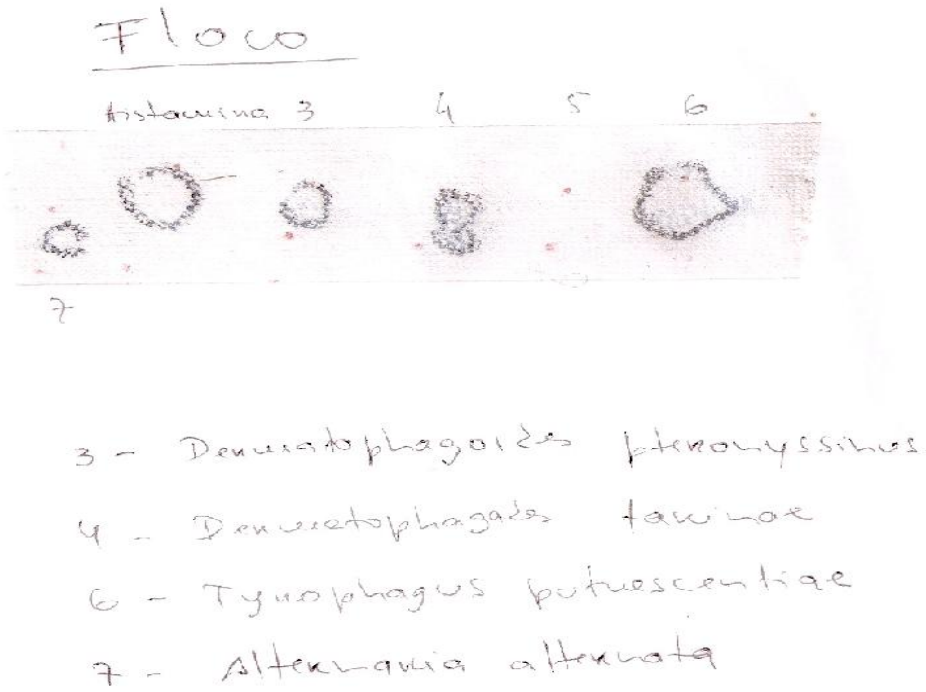

Figure 6. Positive skin prick test results to *D. pteronyssinus*, *D. farinae*, *T. putrescentiae* and *A. alternata* allergens in one atopic dog

Figure 7. Positive SPT and IDT results for *D. farinae* and *D. pteronyssinus* (arrows).

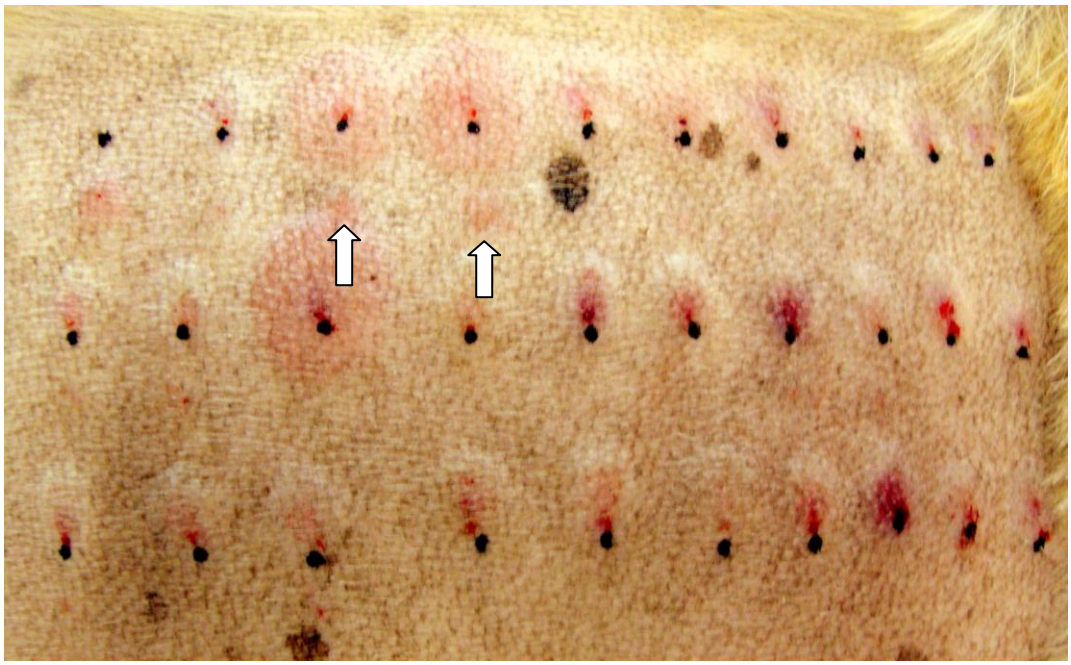

Figure 8. Positive IDT results and negative SPT results for *T. putrescentiae* and *L. destructor* in one atopic dog (arrows).

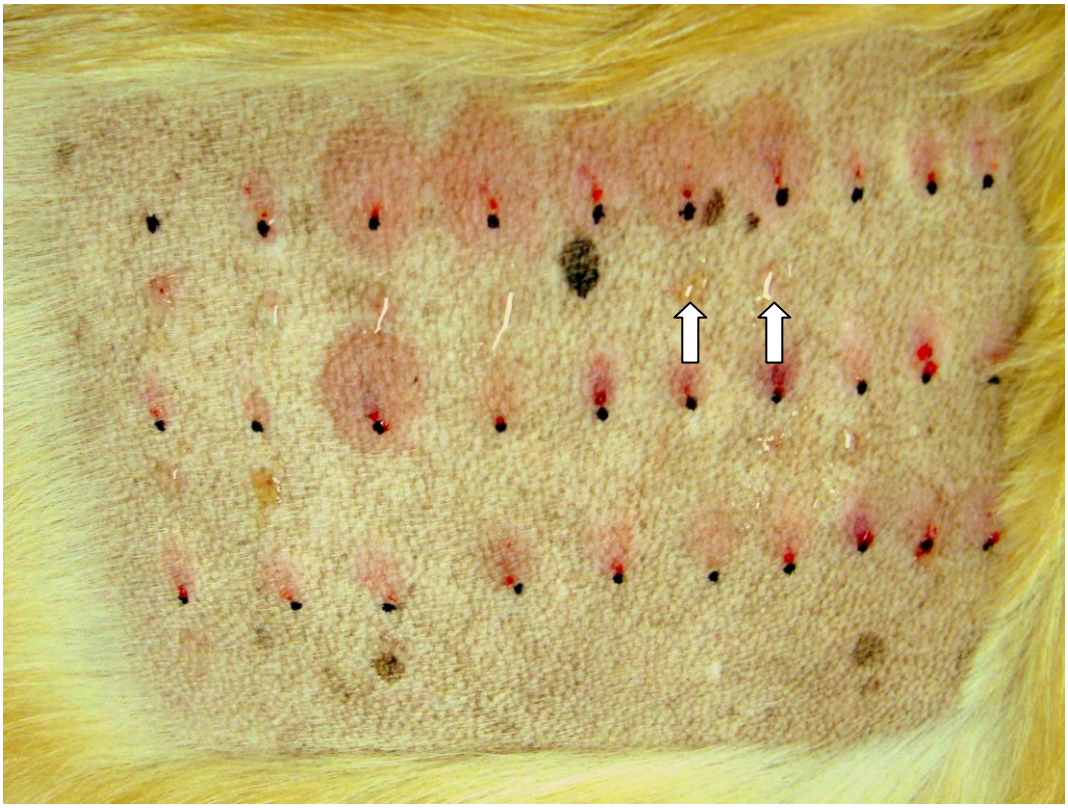

Figure 9. Papule induced by histamine positive control in the skin of the groin in one atopic dog using SPT.

Another possible explanation for these results is the higher specificity of SPT, already shown in human medicine (Demoly et al., 1998; Hillier & DeBoer, 2001). One study, performed in human patients, tried to verify if it would be important to perform IDT, in the presence of a negative SPT, for the diagnosis of grass pollen allergy. It was concluded that a positive IDT response to grass pollens in the presence of a negative SPT response, would not be indicative of a clinically significant sensitivity to these allergens, which proves a higher specificity of SPT. However, this only occurs if sufficiently potent allergen extracts are available for SPT (Nelson, Oppenheimer, Buchmeier, Kordash & Freshwater, 1996).

In our opinion, false positive reactions are at least as worrisome as false negative reactions. In fact if, through allergen-specific immunotherapy, a dog is injected with increasing concentrations of one or more allergens for which is not sensitive, this may induce a clinically significant sensitivity to these allergens (Lourenço-Martins, 2011).

With this study, it was possible to conclude that, in comparison with intradermal tests (IDT), skin prick tests (SPT) are easier to perform and less painful. This is due to the fact that the lancet only enters 1 mm into the skin. To our surprise, most of these dogs did not need to be sedated, as they tolerated very well this procedure not showing any signs of pain or discomfort, in contrary of what happens in IDT. Of the 22 healthy non-atopic dogs, only one required sedation, when using only SPT, due to anxious character. Therefore, in anxious dogs, it may be required to do this procedure under sedation of the animal. It would be

interesting in the future to see if cortisol levels, which may rise due to pain/discomfort or anxiety, would have an influence in the results of SPT. The four atopic dogs used had to be sedated due to the performance of IDT, in conjunction with SPT. It is also shown in this study that the technique of SPT is doable in dogs, as it was possible to confirm the simplicity, rapidity, less discomfort, less irritation, and safety of this method. It is also important to refer that none of the dogs showed any systemic adverse reaction, and the only local side effect observed was minor pruritus and erythema in the positive control (histamine) injection site.

Several factors have been shown to influence the degree of pain or discomfort induced by SPT in children, particularly, the type of lancet used, a previous traumatic experience, the child's age or cultural factors, amongst others seem to have some influence (Duarte et al., 2010). There are two types of validated scales to evaluate the intensity of pain triggered by several procedures, in human medicine, adapted to the various age groups, which include self-assessment and hetero-assessment scales. Self-assessment scales consist in the evaluation of pain by the person experiencing it. These are adequate for children over 3-5 years of age. Hetero-assessment scales consist in the evaluation of pain through the observation of behavioral and physiological markers, by the care provider. They are suitable for newborns, for instance. In children, one of these hetero-assessment scales is OPS (objective pain scale), modified to omit blood pressure measurement, in a score from 0-8, to evaluate children between 2-5 years old, which includes the observation of the body language and posture of the child. In one study, in which the objective was to evaluate the pain caused by SPT in a pediatric population, using several scales to evaluate the pain in children between 2-12 years old, it was concluded that the intensity of the pain was low, indicating that the children only experienced mild pain with this method, and also 15 minutes after the procedure the median pain score was zero, indicating that the pain was transitory (Duarte et al., 2010). It would be interesting in the future to use similar hetero-assessment pain scales in dogs, to evaluate the pain experienced by dogs with the SPT technique.

There is one study performed in horses, where SPT were used to diagnose sensitivity to several aeroallergens commonly involved in equine recurrent airway obstruction (RAO), such as mites, molds, pollens and epithelia. In this study it was concluded that all horses with RAO had several positive SPT reactions to these allergens, which suggests the role of a hypersensitivity mediated by IgE, present in horses with RAO. SPT are the method conventionally used in human medicine to detect the presence of specific IgE in the surface of mast cells of the skin, being one of the most efficient methods, which have good correlation with other tests. Also, it was possible to conclude that the concentrations available for SPT in human medicine are potent enough to induce positive reactions in horses (Tilley et al., 2010).

Also, in the study by Tilley et al. (2010), a *cut-off* value for the wheal diameter in horses has been proposed to be 1 cm, which is significantly higher than the *cut-off* value of 0.3 cm for

the wheal diameter that the World Allergy Organization gives for SPT in human patients. Currently, that the author is aware of, there are no studies for the *cut-off* value in dogs, however, it seems that it is more similar to that suggested for humans (0.3 cm) than for horses. This could be explained by the fact that horses are animals which tend to mount very eosinophilic reactions, developing a more exuberant allergic inflammation towards the allergens tested.

It has been possible to conclude as well that, in horses, SPT results are available immediately, so the clinician is able to show a cutaneous reaction to owners that are hard-to-convince, and have lower costs. Although horses show little discomfort during SPT, unlike dogs, all horses have to be sedated.

Contraindications of SPT in horses include the presence of extensive skin diseases, that do not leave an area wide enough to perform the tests, the risk of anaphylaxis, particularly to some food allergens, or the absence of a trained clinician (Tilley et al., 2010). A more extensive study is needed to prove if this is also true for SPT in dogs. It seems logical to assume contraindications for SPT in dogs should be the same as those for IDT.

In conclusion, potential limitations of our study are:

- The fact that the allergen extracts are standardized for human patients, so it was not possible to acquire higher concentrations for each allergen, which means that it is possible that these concentrations are not potent enough to induce clinically significant positive skin reactions in atopic dogs;
- This technique has not yet been described for use in dogs. It has been recently described for use in horses (Tilley et al., 2010), however these authors did not titrate the concentrations;
- The number of allergens available for the performance of SPT was limited, and so it was not possible to perform an absolute study with the wide battery of allergen extracts available for IDT. This limitation, however, does not seem relevant for the preliminary study of the SPT technique;
- Relative small number of non-atopic dogs used study. Although, 22 healthy non-atopic dogs have been used to test the concentrations available, which was more than the numbers used by other authors, as a control group (10), in a similar study in horses (Tilley et al., 2010).

In order to confirm if the results of SPT have correlation with the results of IDT in atopic dogs, further studies are needed, with a greater number of atopic dogs, sensitive to different allergens (mites, molds, pollens, amongst others), using both methods of skin testing, IDT and SPT. That would be the third phase of this study, however it was not possible to complete this phase due to time constraints.

In the near future, it is important to provide guidelines for the realization of the SPT technique in dogs, describe the advantages and limitations of this technique and define *cut-off* values

for the wheal diameter in dogs, in order to establish a standardized technique for SPT in dogs so that it may be a good method of allergy testing, eventually replacing IDT, just as in human medicine. In order for this to be achieved, further studies are needed.

One thing that could be done is to test other body areas, besides the lateral thorax, such as, the ventral abdomen or the axillae, for instance. This could be interesting because testing in other areas could lead to better results, and also, it would be better in a esthetical point of view, and maybe the owners would be more easily convinced to perform the tests in their dogs.

Knowledge of the pharmacological withdrawal times for the realization of SPT, so that systemic and/or topical therapy does not interfere with the results, should also be studied, which may or not be similar to those used for IDT.

Another thing that could be done in the future is to, by using the concentrated extracts available for intradermal testing, create dilute solutions, so that the concentrations of these solutions are 10 to 100 times those used for IDT and compare these extracts with those provided by ALK-Abelló, commercially available in Portugal, and verify whether it would have positive and concurrent results for those allergens, using skin prick testing. Eventually, it would also be interesting to compare these results with those of serological tests.

As already performed in horses by Tilley et al. (2010), it would be interesting to evaluate whether or not clinical improvement would be present, with allergen avoidance measures, as well as, with allergen-specific immunotherapy in dogs, based on the results of SPT.

Also, in the future, it might be interesting to study the pain experienced by dogs, using subjective or objective hetero-assessment pain scales, similar to those used in children between 2-5 years of age (for obvious reasons, it would not be possible to use auto-assessment pain scales). The subjective scale would include the observation of the animal behavior by the performer of the SPT or by the owner. The objective scale could be used by measuring the blood pressure during the procedure or by the measurement of cortisol levels before and after the procedure, which would also give the information if the cortisol levels were too high to interfere with the results of SPT.

4. Conclusion

Through this study it is possible to conclude that:

1. The SPT technique is doable in dogs;
2. Compared to the IDT technique, it is simpler, more rapid, less distressful, as apparently seems to be less painful, and safer (has a low risk for severe adverse reactions);
3. The standardized allergen concentrations used for SPT in human medicine do not induce irritant false positive skin reactions in healthy non-atopic dogs.

However, further studies are needed in order to conclude if SPT should be recommended to use in skin allergy testing instead of IDT, as it has been done in human medicine for over 30 years.

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